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Section I.

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Heartwater in Sheep.—The Weil-Felix reaction and an investigation into the bacterial content of the blood with particular reference to the use of "K" medium.

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THE WEIL-FELIX REACTION.

The existence of a common antigenic factor [Castanida and Zia (1933), White (1933)] in *Rickettsia* and *Bacillus proteus* X is known to be responsible for the specific O agglutination of *B. proteus* X by typhus serum, originally described by Weil and Felix and now known universally as the Weil-Felix reaction.

Attention was again focussed on this reaction when Felix and Rhodes (1931) confirmed the observation of Fletcher and Lesslar (1925) that there existed two serologically distinct types of tropical typhus which reacted specifically with *B. proteus* X19 on the one hand and *B. proteus* XK on the other. An additional interesting feature of this finding was that *B. proteus* XK was merely a non-indologenic variant of X19, and had been obtained inadvertently during the course of routine subcultivation.

Further investigation into the serological types of typhus virus has led Felix (1933) to correlate " the agglutinogenic and immunogenic properties of different types of virus ". This has led to the suggestion that there is a variant of *B. proteus* X that corresponds serologically with each of the *Rickettsiae* causing the many different diseases of the typhus group.

Cowdry (1926) showed that a Rickettsia, *R. ruminantium*, is the causative agent of heartwater, and consequently the possibility of demonstrating a positive Weil-Felix reaction with one or other of the *proteus* OX strains was considered, since the establishment of such a reaction would be of the greatest value in the further study of the disease.

Our colleague, Dr. E. M. Robinson, informed us (personal communication) that previously he had investigated this reaction in heartwater-affected and -recovered sheep but with negative results. Nevertheless we decided to take up the problem anew using—

(*a*) A larger number of sera from sheep in various stages of the disease, that is during the reaction, at various periods after recovery and after "hyperimmunization" by several injections of virulent blood.

(*b*) Antigens consisting of single cell cultures of OX2, OX19, and OXK in the hope of the chance isolation of a specific variant as was the case with OXK.

(*c*) Antigens consisting of cultures of organisms isolated from sheep infected with heartwater.

(*a*) The initial *proteus* strains used were cultures of OX2, OX19, and OXK, received through the courtesy of Dr. A. Felix, to whom we wish to express our thanks. To serve as controls to these cultures we prepared agglutinating sera in rabbits by repeated intravenous injections of saline suspensions killed by heating at 60° C. for half an hour, and in goats by intravenous injections of saline suspensions of single cell cultures killed with 0.1 per cent. formalin. Cross agglutination tests were carried out with living suspensions in saline of approximately the same density; the volume of fluid in each tube was made up to 2.0 c.c. with saline and readings were taken after two hours at 45° C. and overnight storage at room temperature (22°-26° C.). The results are recorded in Table I.

TABLE I.

Antigens.	Agglutinating Serum.					
	OX2 (s.c.)	OX19 (s.c.)	OXK (s.c.)	OX2 (orig.)	OX19 (orig.)	OXK (orig.)
OX2 (s.c.)...	1,280 (3)	40 (2)	80 (3)	1,280 (3)	80 (1)	20 (1)
OX19 (s.c.)	20 (1)	10,240 (3)	40 (2)	20 (0)	2,560 (3)	20 (0)
OXK (s.c.)..	40 (1)	40 (1)	10,240 (2)	20 (0)	20 (0)	5,120 (2)
OX2 (orig.).	2,560 (1)	40 (2)	80 (1)	1,280 (3)	20 (1)	20 (1)
OX19 (orig.)	40 (0)	10,240 (1)	80 (1)	20 (1)	5,120 (2)	20 (0)
OXK (orig.)	40 (1)	40 (2)	10,240 (2)	20 (0)	20 (0)	10,240 (2)

s.c. = single cell culture. orig. = original culture.

(3), (2), etc. = degrees of agglutination, (3) being complete. Double the dilution noted was negative or trace, and half was complete (3). The lowest dilution used was 1/20.

(*b*) *Agglutination of Single Cell strains of* Proteus *OX*.—Mention has been made of the chance isolation of a serologically distinct mutant of OX19, which reacts specifically with the sera of scrub typhus patients. By the use of this variant OXK and OX 19 scrub typhus may be differentiated from shop typhus, two types of the tropical disease encountered in Malaya and the Dutch East Indies. Therefore it was considered possible that by isolating

from the original *proteus* cultures a large number of single bacilli a variant might be obtained which would be agglutinated by heartwater sera, the assumption being made that there was present an agglutinable variant, but in such small numbers that its agglutination was marked by the much greater number of non-agglutinable organisms. It was realized that if such a variant did exist its isolation would be a matter of pure chance unless many thousands of single-cell cultures were examined. A compromise was made by obtaining 50 single cell cultures from OX2, OX19, and OXK. The resulting 150 antigens were tested against normal sheep sera and heartwater immune sera.

None was agglutinated by any serum at a dilution of 1-40.

(*c*) *Attempt to Isolate a* Proteus *X or other Serologically Similar Organisms from Heartwater Sheep.*—It is the routine practice at these laboratories to maintain the virus of heartwater by passage through sheep. Ample material was available therefore for an attempt to isolate a specific *proteus* or other organism which might have some relation to the heartwater virus. Other authors [Anigstein (1933), Kuczynski (1927), and Martin (1931)], working with diseases of the typhus group, have been able to isolate from various patients strains of *proteus* X which have been shown to have a definite relation with the disease in question.

Kendall (1931) reported that, by the use of his medium, non-filterable bacteria had been rendered filterable, and, further, the inoculation of this medium with apparently bacteriologically sterile blood from influenza patients had resulted in the isolation of a coccus which possessed a definite relation to the virus. We were further stimulated in our investigation by the report of Hadley, *et alia* (1931), in which Shiga's dysentery bacillus was shown to have a filterable stage.

Our main object in undertaking the work was in the hope of isolating a germ which would be agglutinable by heartwater serum, or which could be used as an antigen in the prevention of the disease. As our results were entirely negative, we consider it necessary to record only the main points, omitting details.

METHODS.

In all, material from 51 sheep was investigated—of these 25 were infected with heartwater, 13 with blue tongue, 5 with enteritis [cause unknown (?) dietetic], and 8 were normal animals. All were Merinos, the majority castrated males and the ages varied from 1 to 5 years. The blood of 20 heartwater sheep (87 bleedings), 8 normal sheep (17 bleedings), 13 blue tongue sheep (25 bleedings), and of 5 "enteritis" sheep (5 bleedings) was cultured. In 11 heartwater sheep, scrapings from the jugular vein were cultured, and in four instances the urine. In some cases the blood, jugular scrapings, and the urine from the same sheep were investigated.

Collection of Blood.

Prior to commencing the investigation, an attempt was made to work out a method of obtaining blood in a sterile manner. The method used is given by Mason (1934), and, in brief, consisted in

closely clipping the wool from the jugular region, the application of absolute alcohol and then ether to this area, the insertion, with one thrust, of the needle into the vein, the allowing of 30.0 c.c. of blood to escape, and finally the collection of the sample in a sterile tube.

Jugular Scrapings and Urine.

The sheep was pithed and with sterile precautions, the jugular vein (cervical portion) exposed, and about 20.0 cm. dissected out. The intima was exposed and thoroughly scraped with a knife, the scrapings being transferred to one or other medium. Urine was obtained direct from the bladder by means of a bulb pipette, aseptic precautions being adopted.

Media.

1. Ordinary beef infusion peptone broth.

2. Clot broth. About 10.0-12.0 c.c. of blood was allowed to clot in a test tube and the serum discarded. Broth was then added to replace this serum.

3. Horse flesh infusion peptone agar, plus 5 per cent. of a mixture of equal parts of sheep serum and sheep haemolysed red cells [Mason (13)]. This medium was used in the large flat-bottomed tubes described by Mason (14), and was always incubated for three days at 37° C. prior to use.

4. Litmus lactose agar in the tubes noted under (3).

5. Kendall (K) medium. Three separate lots prepared from sheep gut according to Kendall, and one sample obtained from Difco were used.

6. "E.B." medium prepared from sheep brain according to Eberson and Mossman (15).

7. Robertson's meat broth (horse flesh) medium.

All media were autoclaved for half-an-hour at 120° C. prior to use. [In the case of medium (3) the agar was autoclaved and having cooled to 45°-50° C. the serum-haemolysed-cells mixture was added.]

General Scheme.

When blood was investigated, bleedings were usually made at the first rise of temperature and then at 1 or 2 days intervals until the death of the animal. When broth was used from 0.5 c.c. to 1.0 c.c. of blood was pipetted into 10.0 c.c.-15.0 c.c. of medium, and this incubated at 37° C. for 14 days; a sub-culture was then made into fresh broth, this incubated for a further 7-14 days, and finally a further sub-culture made on serum-haemolysed-cells-agar or Hauduroy's (19) serial plating method on either serum-haemolysed-cells-agar or litmus lactose agar was carried out.

Blood clots were incubated at 37° C. for one month (reduction in volume due to evaporation being made up with sterile distilled water), when a smear was made on serum-haemolysed-cells-agar, and this incubated for 48 hours.

K medium (30.0 c.c.-50.0 c.c. with 0.5 c.c.-1.0 c.c. of blood) was incubated at 30° C. or 37° C. for 14-28 days. Two further subcultures (0.5 c.c.-1.0 c.c. into 10.0 c.c.-15.0 c.c. medium) were made at 10-14 days interval. If no growth was demonstrable (either by naked eye or by stained smear) Hauduroy's serial plate procedure on serum-haemolysed-cells medium or litmus lactose agar was adopted, from 3-8 such platings being conducted.

Throughout, smears stained by Giemsa's and Gram's methods were examined even in apparently negative tubes.

Special attention was given to any tube, which, by stained smear examination, showed what could be developmental forms of bacteria. Serial platings of such material were carried out as many as 25 times before a negative result was accepted.

Every micro-organism which grew, with the exception of obvious contaminants from the sheep's skin or from the air was investigated. This consisted in (1) carrying out with every germ agglutination tests with the heartwater sera previously mentioned, (2) in some cases injecting a K culture or a saline suspension of the germ intracerebrally and intraperitoneally and sometimes intratesticularly into guinea pigs and mice and subinoculating the brains of such animals intracerebrally into fresh guinea pigs and mice, and (3) injecting living K culture and saline suspensions of the microbes subcutaneously and intravenously into sheep and later testing with virulent blood for immunity against heartwater.

Organisms which developed quickly, within 1-3 days, in the original culture tube, and which on further examination proved to be aerobic Gram positive spore-forming bacilli, staphylococci, large copiously growing aerobic Gram positive bacilli (usually pigment producers) were considered to be contaminants and were discarded. All diphtheroids (of which a considerable number was isolated) and Gram negative bacilli were retained and examined in some detail; in addition to the tests already mentioned, the Gram negative bacilli were investigated biochemically.

Results.—These may be briefly summarised under five headings:—

(1) No *proteus* X-like bacillus was isolated.

(2) No germ, which was isolated, was agglutinated by heartwater or *proteus* OX sera at a titre significantly higher than that obtained with normal serum.

(3) It was not possible to produce a transmissable disease, symptom or reaction in guinea pigs or mice by the intracerebral, intraperitoneal or intratesticular injection of living K culture or saline suspension of 8 organisms obtained from 6 sheep.

(4) K cultures and saline suspensions of 14 germs from 12 sheep injected intravenously and subcutaneously (simultaneously) into 28 sheep, apart from a transitory rise in temperature, produced no reaction. Sheep, treated with two such inoculations at from 2-3 weeks interval, were not immune to heartwater, when tested 10-20 days later with intravenous injections of virulent blood.

(5) Formol-killed saline suspensions of *proteus* OX2, OX19 and OX2 (two subcutaneous injections at 14 days interval) did not immunise sheep against heartwater (as tested by the intravenous injection of virulent blood).

Discussion.

In Appendix 1 a résumé is given of the treatment adopted with each sheep and of the germs isolated therefrom. It will be noted that from the heartwater sheep the following germs were isolated: 22 diphtheroids (small Gram positive rather sparsely growing bacilli), 11 staphylococci (white or yellow), 7 Gram negative bacilli (*B. cloacæ*, *B. pyocyaneus*, a member of the alkaligenes group, and three unidentified bacilli, not members of the colontyphoid or *proteus* groups), two tetrads, two streptococci, and 10 Gram positive copiously growing aerobic bacilli (8 sporers), and one Gram negative coccus. From the 26 remaining sheep (normal, blue tongue and "enteritis") four diphtheroids, one staphylococcus and one Gram positive aerobic sporer were obtained. Although the number of bleedings is not large enough to lend itself to accurate statistical analysis, it is interesting to note that basing results on the number of bleedings, 6 of 37 (16.2 per cent.) of the non-heartwater bleedings gave positive growth results, and 55 of 87 (63.2 per cent.) of the heartwater bleedings and jugular scrapings were positive. Reckoning only diphtheroids, the figures become 4 of 37 (11 per cent.) and 22 of 87 (25 per cent.).

We are of the opinion that possibly in non-heartwater sheep the figure 11 per cent. for diphtheroids is actually too high. This is based on results obtained in similar but not identical investigations (unpublished) on the sterility of the blood of normal sheep. These results showed that blood taken direct from the heart of a killed lamb or sheep was sterile; the occasional growth obtained was obviously of aerobic origin.

We are unable to state if the diphtheroids (or other germs) obtained from the blood of heartwater sheep have actually any connection with the virus of the disease. There is no result which would indicate any relation, but the possibility does exist that one or more might have been a developmental stage of the virus, but not at any time obtained by us in a phase capable of being specifically agglutinated or of setting up disease symptoms in laboratory animals or sheep. However, we consider it more likely that they had their origin in the intestine and were able to escape easily into the blood stream through the inflamed gut. This opinion is strengthened by the fact that the highest percentage of positive blood cultures was obtained when the disease was well advanced, bleedings taken prior to the rise in temperature usually being sterile.

One germ only deserves a somewhat detailed description; its growth characteristics, the apparent developmental phase through which it went, and its pleomorphism gave us cause for hope that we had obtained a bacillary stage of the causative rickettsia.

Sheep 34443.—Injected 17/10/32 with 10.0 c.c. of virulent blood from sheep 34910. This latter animal died from and showed the typical lesions of heartwater on 28/10/32. On 24th October,

1932, 1.0 c.c. of blood was sown in 30.0 c.c. of K medium, and incubated at 37° C. After four days the medium was slightly cloudy, but a Giemsa smear showed nothing to arouse suspicion. Sub-cultures on serum-haemolysed-cells-agar, incubated aerobically and anaerobically remained apparently sterile. A Giemsa smear from the original K tube made on 3rd November, 1933, revealed a large number of tiny red-blue bacilli, which when they occurred in clumps, closely resembled *rickettsiae*. Aerobic and anaerobic sub-cultures on agar remained without visible growth. Hauduroy's technique on litmus lactose agar was now adopted and on the third passage (24 hours incubation between each) what appeared to be Hadley's G colonies were obtained. These were so tiny as to be visible only by the aid of a lens when the tube was held in a certain position in relation to the source of light. Giemsa smears showed a very pleomorphic organism; every gradation between a rickettsia-like microbe and a medium-sized bacillus was seen, but the predominant form was what might be termed " knobbly ", i.e. cocci showing protuberances, tapering bacilli ending in swellings or nodular-curved rods.

At this stage the germ would not grow on " dry " serum agar or in ordinary broth, but did so on the surface of " flooded " agar. The serial passages were continued 13 times, at which stage the germ grew reasonably well—sub-cultures on " dry " agar or in broth gave, after 2-4 days' incubation at 37° C., a scanty, rather dry grey growth or a faint turbidity. Smears made at this time revealed a bent or curly Gram positive bacillus, still " knobbly ", and only a very occasional coccus, or rickettsia-like organism. Cultures in K medium, broth, serum-haemolysed-cells-agar and litmus lactose agar made during a period of six months did not result in any further change in morphology or type of growth. The bacillus was not agglutinated by heartwater or *proteus* OX sera at a dilution of 1/40. K cultures (2 and 10 days at 30° C. and 37° C.) and saline suspensions from " flooded " agar injected intraperitoneally, intracranially and intesticularly into guinea pigs caused no disease; the brains of the intracranially injected animals passaged into fresh guinea-pigs (and these later again passaged) gave negative results. The same technique in mice (omitting the intratesticular route) gave the same negative results. As appendix shows, sheep were not demonstrably affected by the intravenous and/or subcutaneous injection of K culture or saline suspensions and were not later shown to be immune to heartwater.

Conclusions.

(1) The H and O variants of *proteus* X2, X19 and XK are not agglutinated by serum from sheep, affected with, recovered from or " hyperimmune " to heartwater.

(2) None of 50 single-cell cultures (150 in all) from the O variants was agglutinated by these sera.

(3) Blood cultures from sheep infected with heartwater gave more positive growth results than did blood from normal sheep or from sheep affected with blue tongue or an enteritis of unknown

origin. A considerable percentage of the organisms isolated probably had their origin in the intestine and escaped via the inflamed gut into the blood stream.

(4) Of the microbes investigated, none was specifically agglutinated by heartwater or *proteus* OX sera. In addition none set up disease symptoms in guinea pigs, mice or sheep, and sheep injected twice with living cultures were not protected against heartwater.

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APPENDIX 1.

("Not agglutinated" means that the organisms were not agglutinated by heartwater or *proteus* OX serum at a dilution of 1-50.)

Sheep 31281.—Bled five times, at daily intervals, during fever. Blood allowed to clot, serum decanted, and clots incubated aerobically and anaerobically. Sub-cultures on serum agar and serum broth put up daily from depth of clot and these incubated aerobically and anaerobically. The first two bleedings sterile; from remainder a streptococcus, large Gram negative bacillus, small Gram negative bacillus, and a diphtheroid isolated. The bacilli not agglutinated.

Sheep 31763.—Bled three times during fever, as clot broths. A staphylococcus, a Gram positive aerobic sporer and a pleomorphic diphtheroid isolated. Last germ not agglutinated.

Sheep 31098.—Bled six times during fever, as clot broths and into meat broth. First two and fifth bleedings sterile. The third, fourth, and sixth bleedings positive in 1-3 days. *B. pyocyaneus*, *B. cloacae*, member of alkaligenes group, Gram negative bacillus (not colon-typhoid or *proteus* group) staphylococcus and a dipththeroid. None agglutinated.

Sheep 32120.—Bled seven times during fever as clot broths. The third, fifth, and seventh bleedings sterile. Bleeding 1 positive 9 days Gram positive sporer; bleeding 4 staphylococcus 14 days; bleeding 6 Gram positive sporer 17 days; bleeding 2 by Hauduroy's technique diphtheroid—not agglutinated and non-pathogenic.

Sheep 32118.—Bled nine times prior to and during fever as clot broths. First, second, fourth, and ninth bleedings sterile. From remainder a staphylococcus and Gram positive sporer. Pithed at height of fever. Jugular scrapings into its own clot broth, clot Tyrode and into clot Tyrode and clot broth from a normal sheep. One (its own) clot broth positive 10 days, Gram positive sporer. Remainder sterile.

Sheep 31720.—Pithed at height of fever. Jugular scrapings into normal sheep clot broth and its own clot broth, and in addition a clot broth put up. All tubes sterile after 14 days. With Hauduroy's technique a fine diphtheroid isolated from one tube. After one month's sub-culturing this grew as a profuse, yellow paint-litle growth. Non-agglutinated.

Sheep 32049.—Bled eight times prior to and during fever. The first three bleedings sterile. From remainder after 3-8 days, a Gram positive sporer, a streptococcus, a staphylococcus and a fine diphtheroid obtained. The diphtheroid not agglutinated.

Sheep 33518.—Bled out at height of reaction. Clot broths made, clot broths inoculated with jugular scrapings and clot broths from normal sheep clot broth inoculated with scrapings. A Gram negative bacillus (not colon-typhoid or *proteus* group), a Gram negative coccus and a Gram positive cocco-bacillus isolated. The first germ not agglutinated.

Sheep 34103.—Pithed at height of reaction. Clot broths made: jugular scrapings into normal sheep clot broth, urine into meat broth and normal sheep clot broth. The normal sheep clot broth plus scrapings positive eight days with diphtheroid. Not agglutinated. Remainder sterile.

Sheep 34607.—Pithed at height of reaction. Jugular scrapings into its own clot broth, urine into its own clot broth, meat broth and broth. Urine-broth, positive three days with staphylococcus, scrapings—clot broth positive four days Gram positive sporer.

Sheep 34103.—Pithed at height of reaction. Jugular scrapings into normal sheep clot broth and urine into broth, meat broth and normal sheep clot broth. A clot broth made from heart blood. Scrapings and urine sterile. Clot broth gave a fine Gram negative bacillus and a fine diphtheroid. Neither agglutinated.

Sheep 34095.—Pithed at height of reaction. Jugular scrapings into meat broth—positive seven days Gram positive sporer, blood into meat broth—positive four days staphylococcus, urine into broth—positive four days staphylococcus.

Sheep 34910.—Bled twice during fever into K medium and into broth. Ks at 30° C., broths at 37° C. First K bleeding sterile (serially passaged on litmus lactose agar nine times; second K bleeding apparently sterile on sixth serial passage. The fifth and sixth passage tubes sealed off and set aside for six weeks at room temperature. A diphtheroid (good growing) growing on fifth tube and a tetrad on the sixth. Neither agglutinated.

The broths treated as the Ks. Both sterile.

Sheep 34054.—Bled six times during fever as 34910.

Ks: First, fourth, fifth, and sixth bleedings sterile. From the second and third a tiny diphtheroid isolated. Not agglutinated.

Broths: The first five bleedings incubated at 37° C. for from 25 to 28 days. Subcultures in K incubated at 37° C. for one month and smears made on agar—all sterile. The sixth bleeding positive three days—staphylococcus.

Sheep 3443.—Bled six times during fever as 34910.

Ks: By mistake, all but one bleeding 24.10.32 (the third) discarded. For description of germ obtained see text.

Broths: From the first three bleedings a staphylococcus, and from the fifth a Gram positive sporer obtained; the fourth and sixth sterile after one month. The positive results were got in the original tubes after 7-14 days incubation.

Sheep 35024.—Bled five times during fever as 34910.

Ks: The second, third, fourth, and sixth bleedings sub-cultured at 14 days interval, four times through K and then serially plated three times; all negative. First bleeding, good growing diphtheroid isolated from original tube after 17 days. Not agglutinated. Fifth bleeding—poorly growing diphtheroid from original tube after 12 days. Not agglutinated.

Broths: Original tubes negative after one month; sub-cultues on agar negative after one week.

Sheep 35029.—(Injected i.v. with 35.0 c.c. of a 24 hours K culture of diphtheroid isolated from the third K tube of 34054. No reaction. After nine days bled into K and broth.)

K: By serial platings a Gram positive, fine granular bacillus isolated. On smear, the organism often appeared to be made up on tiny granules. Not agglutinated.

Broth: Sterile after one month.

Sheep 35007.—Bled thirteen times prior to and during fever as 34910.

Ks: The first to seventh, ninth, eleventh, twelfth, and thirteenth bleedings sub-cultured through K three times at 14-day-intervals. Then serially plated three times—all negative. From the eighth and tenth fine diphtheroids isolated—neither agglutinated.

Broths: All incubated one month and then sub-cultured on agar for three days. All negative, except the second—a large, good growing Gram positive bacillus.

Sheep 35533: Bled seven times during fever as 34910.

Ks: The first, third, fifth, sixth, and seventh bleeding incubated for one month, then serially plated three times—all negative. From the fifth a copiously growing Gram positive bacillus isolated from original tube after four days incubation. From the fourth a fine diphtheroid obtained. Not agglutinated.

Broths: All incubated 25 days—then into fresh broth for 10 days—then on agar—all negative.

Sheep 34894.—(Received 90·0 c.c., intravenously, of a 24 hours K culture of Gram positive bacillus isolated from 34443, 24/10/32. No reaction.)

Nineteen days later bled into K. Sub-cultured into fresh K in 7 days, irto EB in a further 16 days, and 2 days later serial plating commenced. By this technique a rather stout vacuollated "knobbly" bacillus isolated. Grew very poorly. Not agglutinated.

Sheep 34761.—Bled three times during fever as 34910.

Ks: Incubated 20 days, then into fresh K for 7 days—serial platings three times. The second, third, and fourth bleedings sterile. From the first a very profusely growing Gram positive bacillus.

Broths: Incubated one month—then into fresh broth for 10 days—all negative.

Sheep 34177: Pithed at height of reaction and jugular scrapings cultured in K and broth.

K: Positive in 8 days—staphylococcus.

Broth: Incubated for 14 days—then into fresh broth—negative 14 days.

Sheep 35829.—Pithed at height of reaction and jugular scrapings cultured into K and broth.

K: Incubated 10 days; then into fresh K for 10 days; sown on agar—staphylococcus.

Broth: Incubated 10 days; then in fresh broth. Negative 14 days.

Sheep 35035.—Treated as 35829.

K: A "knobbly" diphtheroid isolated. Not agglutinated.

Broth: Negative.

Sheep 34871.—Treated as 35829.

K: A profusely growing Gram positive bacillus isolated. Not agglutinated.

Broth: A staphylococcus isolated.

Normal Sheep.

Seven normal sheep (35803, 35808, 34806, 34998, 34093, 35819 and ×) bled into K and broth as 34910.

Ks: Incubated for 14 days; then sub-cultured into fresh K and incubated for a further 14 days. Then cultured in "flooded" agar for seven days (evaporated fluid replaced as required with sterile distilled water). All negative.

Broths: Incubated for 14 days; then into fresh broth for 14 days. All negative.

Sheep Y: Ten clot broths made at one bleeding. Incubated for one month. Two tubes positive after four days incubation, with apparently the same fine growing diphtheroid.

Blue Tongue Sheep.

Sheep 34681, 34713, 34751.—Bled at height of temperature reaction into meat broth and as clot broths. The first positive in four days—staphylococcus. Remainder sterile after 20 days' incubation.

Sheep 34562, 34633, 34750, 34581, 34522, *and* 34626.—Bled as clot broths at height of temperature reaction. The first positive in 10 days—fine diphtheroid. Remainder negative after 20 days.

Sheep 35007, 35003, 35033, 35006.—Bled during fever on each of three successive days into meat broth and broth. All incubated 20 days. The first positive (in meat broth only) with a fine growing diphtheroid.

Sheep 35001, 35035, 35011.—Bled during fever into broth and meat broth. All negative after one month's incubation.

"Enteritis" Sheep.

Sheep 34356, 34547, 34717, 34653, *and* 33961.—Bled as clot broths and into broth. The first positive with a Gram positive sporer after four days. Remainder negative in 20 days.

APPENDIX 2.

Summary of the inoculations of living cultures and suspensions into sheep with the result of the immunity test with virulent heartwater blood:—

Sheep.		*Treatment.*	*Immunity Test.*
32957	22/3/33	10.0 c.c. s.c. saline suspension of fourth bleeding diph. of 35533	18/4/33 died H.W.
	5/4/33	25.0 c.c., ditto.	
32952	22/3/33	10.0 c.c. s.c. saline suspension of diph. of 34443	Do.
	5/4/33	20.0 c.c., ditto.	
32958	22/3/33	10.0 c.c. s.c. saline susp. of Gram pos. bacillus of 34871	Do.
	5/4/33	20.0 c.c., ditto.	
32949	22/3/33	10.0 c.c. s.c. saline susp. of diph. of 35035	Do.
	5/4/33	20.0 c.c., ditto.	
33117	22/3/33	10.0 c.c. s.c. saline susp. of staphylococcus from 34177	Do.
	5/4/33	20.0 c.c., ditto.	
33118	22/3/33	10.0 c.c. s.c. saline susp. of staphylococcus of 35829	Do.
	5/4/33	20.0 c.c., ditto.	
32948	22/3/33	10.0 c.c. s.c. saline susp. of gram pos. bacillus of 35029	Do.
	5/4/33	20.0 c.c., ditto.	
32507, 33119, 33104	22/3/33	10.0 c.c. s.c. of formol-killed saline susp. of OX2, OX19, and OXK respectively	All died H.W.
	5/4/33	20.0 c.c., ditto.	
35803	9/1/33	2.0 c.c. i.v. 15.0 c.c. s.c. saline susp. of bacillus of 34894	15/2/33 died H.W.
	27/8/33	5.0 c.c. i.v. 15.0 c.c. s.c. saline susp. of bacillus of 34894.	
35808	9/1/33	2.0 c.c. i.v. 10.0 c.c. s.c. saline susp. of first bleeding diph. of 35024 ...	15/2/33 died H.W.
	23/1/33	5.0 c.c. i.v. 20.0 c.c., ditto.	
35819	9/1/33	2.0 c.c. i.v. 95.0 c.c. s.c. saline susp. diph. of 34443	Do.
	23/1/33	5.0 c.c. i.v. 17.0 c.c. s.c. saline susp. diph. of 34443.	
34550	9/1/33	2.0 c.c. i.v. 20.0 c.c. s.c. of 10 days (37° C.) K. culture of bacillus of 34894	Do.
	23/1/33	5.0 c.c. i.v. 25.0 c.c. s.c., ditto.	
	1/2/33	5.0 c.c. i.v. 35.0 c.c. s.c., ditto.	
34480	3/1/33	10.0 c.c. s.c. saline susp. of diph. of second bleeding of 34054	15/2/33 died H.W.
	23/1/33	25.0 c.c. i.v., ditto.	
34576	9/1/33	2.0 c.c. i.v. 45.0 c.c. of 10 days K culture (37° C.) of diph. of 34443 ...	Do.
	23/1/33	5.0 c.c. i.v. 27.0 c.c. s.c. of 10 days K culture (37° C.) of diph. of 34443.	
34891	9/1/33	2.0 c.c. i.v. 20.0 c.c. s.c. 10 days (37° C.) K culture of diph. of first bleeding of 35014	Do.
	23/1/33	5.0 c.c. i.v. 25.0 c.c. s.c., ditto.	
32109	9/1/33	2.0 c.c. i.v. 15.0 c.c. s.c. of saline susp. of diph. of fifth bleeding of 35024 ...	Do.
	23/1/33	5.0 c.c. i.v. 20.0 c.c. s.c., ditto.	
34390	9/1/33	2.0 c.c. i.v. 25.0 c.c. s.c. 10 days (37° C.) K culture of diph. of fifth bleeding of 35024	Do.
	23/1/33	5.0 c.c. i.v. 20 c.c. s.c., ditto.	

Sheep.		*Treatment.*	*Immunity Test.*
34420	9/1/33	2.0 c.c. i.v. 15.0 c.c. s.c. saline susp. of diph. of 34910	Do.
	23/1/33	5.0 c.c. i.v. 15.0 c.c. s.c., ditto.	
35001	3/1/33	10.0 c.c. s.c. saline susp. of diph. of 34054	Do.
	23/1/33	20.0 c.c. s.c., ditto.	
33577	9/1/33	10.0 c.c. i.v. 125.0 c.c. s.c. of 10 days (37° C.) K culture of diph. of 34054	Do.
	23/1/33	5.0 c.c. i.v. 15.0 c.c. s.c., ditto.	
34871	28/12/32	33.0 c.c. i.v. 4 days (37° C.) K.B. culture of diph. of 34894	26/1/33 died H.W.
34281	12/12/32	25.0 c.c. i.v. 6 days (30° C.) K culture of diph. of 34910	4/1/33 died H.W.
35031	29/11/32	17.0 c.c. i.v. 4 days (37° C.) K culture diph. of 34054	20/12/32 died H.W.
34894	9/11/32	90 c.c. i.v. 24 hours K culture of dipth. 34443	29/11/32 reacted. H.W. recovered.
34290	16/11/32	33.0 c.c. i.v. 24 hours K culture of diph. of 34054	20/12/32 died H.W.
35029	16/11/32	35.0 c.c. i.v. of 24 hours K culture diph. of 34054	20/12/32 died H.W.
35006	26/11/32	20.0 c.c. i.v. saline susp. of tetrad of 34910	29/12/32 died H.W.
35034	5/12/32	25.0 c.c. i.v. 4 days (30° C.) K culture of diph. of 34443	29/12/32 died H.W.
35026	12/12/32	25.0 c.c. i.v. 6 days (30° C.) K culture of tetrad of 34910	4/1/33 died H.W.

i.v. =intravenous injection.
s.c. =subcutaneous injection.
susp. =suspension.
diph. =diphtheroid.
pos. =positive.

NOTE.—Where repeated injections were given, fresh saline suspensions or fresh K cultures were injected. The saline suspensions were prepared by washing off the growths from the surface of agar (or "flooded" agar) after 24 to 48 hours' incubation. The immunity tests were arranged so that the routine virus-passage sheep served as controls. All of these died of or reacted with heartwater, but owing to the negative nature of the experiment have not been included in the appendix.

Section II.

Parasitology.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 3, Number 1, July, 1934.

A Search for Tick Parasites in South Africa.

By R. A. COOLEY,† Entomologist, U.S. Public Health Service, Hamilton, Montana.

WITH over 300 species of ticks (*Ixodidae*) known and with the increasing number of diseases of man, domestic animals, and wild animals, known to be carried by ticks, it is only natural that there should be a general interest in the possibility of biological control. This led to a visit to South Africa by the writer in 1928, to search for tick parasites, especially for such species as might be of value for the control of *Dermacentor andersoni* Stiles. This tick is a serious problem in the Rocky Mountain region of the United States where it transmits Rocky Mountain spotted fever and other infections to man, and is concerned in the transmission or causation of several diseases of animals.

Searching for tick parasites is relatively new in the experience of entomologists. In the field of agricultural entomology there are thousands of known parasites attacking insects that feed on plant life, and various workers have spent much time in looking for new species in other countries for use in their home lands. These entomologists have had the guidance of experience and the writings that have accumulated through many years. In the field of medical entomology, relatively few parasites of disease transmitting or disease causing insects, and only two parasites of ticks, are known. These are *Ixodiphagus texanus* Howard (17) and *Hunterellus hookeri* Howard (18) which are closely related phylogenetically and very similar in their biologies. *Ixodiphagus caucurtei* du Buysson (12) described from France has recently been determined to be synonymous with *H. hookeri.*

In the attempted biological control of *D. andersoni* Stiles in the United States, as carried on by the Montana State Board of Entomology from 1926 to July 1, 1931, and by the Bureau of the U.S. Public Health Service, since that time, the writer has had experience with the tick parasites referred to above and before leaving for Africa had assembled several trained assistants, who were on duty while he was away. They were thus in a position to receive and make use of any new parasites that might be found.

In view of the rather extensive and thorough faunal surveys of ticks that had been made by various workers in the United States, and particularly by the U.S. Bureau of Entomology of the Department of Agriculture, it was believed that we probably had discovered

* This report has been prepared as a co-operative project between the Bureau of the United States Public Health Service and the Montana State Board of Entomology.

† Resigned 1st July, 1931, as Professor of Entomology at Montana State College, State Entomologist, and Secretary of the Montana State Board of Entomology, and became Entomologist, Bureau of United States Public Health Service, Rocky Mountain Spotted Fever Laboratory, Hamilton, Montana, U.S.A.

in America all the tick parasites present in the native fauna. In arriving at this opinion we were influenced by some knowledge of the methods that were used in the faunal surveys that had been made. In many instances the entomologists had kept the living ticks under observation and allowed the fed larvae and fed nymphs to develop and emerge into the next stage. An opportunity was thus afforded for detecting any tick parasites present, excepting latent ones. The latter could be found only by a further feeding of the immature ticks in the laboratory. Therefore it appeared to be more desirable to make search for parasites on some other continent rather than in the United States.

Africa was selected for the search for several reasons. There is present there an extensive tick fauna and most of the species are indigenous. G. A. H. Bedford (2) has recently listed 61 species in South Africa alone. It has been less disturbed by agriculture, industry, and commerce, than have the other continents, and the fauna has evidence of having gone through extensive and profound biological changes in the course of evolution. Ticks must have been present for ages and they must have afforded an opportunity for the adaptation of parasites in ticks with diverse habits. True, there are other regions where ticks have existed since very early times, but it seems clear that there are present on the continent concerned, many well adapted, or specialized ticks, which should afford opportunity for further specialization of parasites that attack them. This is illustrated by the discovery by Bedford (1) of the remarkable tick, *Nuttalliella namaqua* which appears to be intermediate between *Argasidae* and *Ixodidae*. It is his opinion that the finding of this tick "seems to indicate that the *Ixodidae* may have originated in Africa".

Further, it was hoped that there might be found in South Africa, localities where some specialized tick could be discovered in relatively few numbers where parasites might be the cause for the lesser numbers. South Africa was chosen for the venture because it is remote from the equator, and the climate resembles more closely that found where *D. andersoni* is adapted in America.

The writer was pleased to be able to work under the auspices of the Government of the Union of South Africa at the Laboratories of Veterinary Services and Animal Industry at Onderstepoort which, in both accomplishment and facilities, stand very high among similar institutions of the world.

A thorough search for tick parasites in Africa, even in the southern part of the continent alone, would have required more time than was available and it was necessary to adopt a method that would lead directly to a maximum of results. The simple method employed was to secure numerous ticks from as many kinds of animals as possible. It was desirable also to get the ticks from many different localities. The ticks taken were kept alive under observation for parasitism. According to previous experience, about two-thirds engorgement is necessary either to permit the parasites to mature or to insure the development of the tick to the next stage. It was realized also that most of the different species of ticks available in the country could be obtained in the better settled agricultural regions, on domestic animals and the small wild mammals. It seemed to be

desirable also, to collect ticks from large wild animals and from the smaller mammals in the more remote regions where, it was believed, that in the more natural environment there might be a greater possibility of finding parasites.

The phenomenon of latency in *Hunterellus hookeri* had been discovered (9) just before departing for Africa, and its possible occurrence in any parasite was apparent. The method used as outlined above, would not bring to light any latent parasites that might be present, for in order to detect them it would be necessary to go through further procedure including the feeding of the ticks in the laboratory. This would have required not only more time, but trained helpers, further equipment, and a variety of laboratory animals.

RECORD OF TICKS COLLECTED IN SOUTH AFRICA.

It is not necessary to give in detail the various collecting trips that were taken. All of the collecting done is summarized in the following paragraph.

The Morning Market at Pretoria was visited for a few days beginning July 14, for the purpose of examining hares and some of the larger game animals. Later a man employed for the purpose, continued these observations. Brief collecting trips were made on roads leading out of Pretoria whenever there was opportunity. More extended trips were taken to Warmbaths, Pienaar's River, and to the vicinity of Hartebeestpoort Dam. Other trips were taken in the vicinity of Messina and along the Limpopo River and near the Kruger National Park Game Reserve, where collecting was done on lands owned by the Transvaal Consolidated Lands and Exploration Company. Mr. C. P. Lounsbury, a former college mate in Massachusetts, U.S.A., made an extended automobile tour through the southern Transvaal, Orange Free State and Cape Province, and secured for the writer, at no expense to him other than the petrol used, a large number of ticks.

Because of their nocturnal habits it was easier to secure certain of the small animals at night, such as hares, spring hares and gennets, etc. Most of the animals were shot. The desirability of capturing animals by traps in order to afford an opportunity to hold them in cages while any infesting ticks fed to repletion, was fully recognized. Only a small amount of trapping was possible, however. Mice were easily secured, but it was soon learned that they carried no ticks.

It should be realized that since the collecting was extended only from June 4 to October 17, the writer did not have the full opportunity to find any possible tick parasites. To be most effective such a survey should be continued through a full year in each general locality studied.

The locality of each tick lot taken in all of the collecting is given with dates in the tabulation below. The entire collection of ticks was examined by Dr. G. H. F. Nuttall and Mr. Cecil Warburton of Cambridge, England, and the writer desires to express his appreciation of their kindness in naming them.

SOUTH AFRICAN TICK RECORDS.

1928.	Ticks.	Parasites.	Hosts.	Localities.
4th June	*R. evertsi* Neum. *B. decoloratus* Koch	—	Cattle	Capetown, C.P.
4th June	*B. decoloratus* Koch	—	Cow	Capetown, C.P.
14th June	*H. aegyptium* Linn. *B. decoloratus* Koch	*	Hare, *Lepus zuluensis* Thos. and Schw.	Pretoria, Transvaal, Morning Market.
20th June	*B. decoloratus* Koch	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Morning Market.
20th June	*H. aegyptium* Linn. *R. oculatus* Neum.	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Morning Market.
23rd June	*R. oculatus* Neum.	—	Horses	Pretoria, Transvaal.
23rd June	*H. aegyptium* Linn.	—	"Dikkop" *Burhinops capensis* Lcht. (a bird)	Pienaar's River, Transvaal.
25th June	*R. appendiculatus* Neum. *R. evertsi* Neum. *A. hebraeum* Koch *H. aegyptium* Linn.	— •	Cattle	Warmbaths, Transvaal.
26th June	*Hyalomma* sp. *R. appendiculatus* Neum.	—	Horses	Pretoria, Transvaal.
30th June	*H. aegyptium* Linn.	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Morning Market.
30th June	*R. oculatus* Neum. *H. aegyptium* Linn.	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Morning Market.
4th July	*H. aegyptium* Linn.	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Morning Market.
28th June	*R. evertsi* Neum.	—	Kudu, *Strepsiceros strepsiceros* Pallas	Grahamstown, C.P.
7th July	*R. evertsi* Neum.	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Morning Market.
6th July	*H. aegyptium* Linn. *R. oculatus* Neum.	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Morning Market.
6th July	*R. evertsi* Neum.	—	Sable antelope, *Ozanna nigra* Harris	Messina, Transvaal, Dongola Farm.
7th July	*R. evertsi* Neum. *B. decoloratus* Koch	—	Kudu, *Strepsiceros strepsiceros* Pallas	Messina, Transvaal, Dongola Farm.
8th July	*Boophilus* sp.	—	Hare, *L. zuluensis*	Messina, Transvaal, Dongola Farm.
7th July	*R. evertsi* Neum. *H. aegyptium* Linn.	—	Roan antelope, *Equinus equinus* Desm.	Messina, Transvaal, Dongola Farm.

6th July	*H. aegyptium* Linn. *B. decoloratus* Koch	—	Hare, *L. zuluensis*	Messina, Transvaal, Dongola Farm.
8th July	*B. decoloratus* Koch *R. evertsi* Neum	—	Duiker, *Sylvicapra grimmi* L.	Messina, Transvaal, Dongola Farm.
6th July	*R. evertsi* Neum. *B. decoloratus* Koch	—	Impala, *Aepyceros melampus* Lcht.	Messina, Transvaal, Dongola Farm.
10th July	*R. oculatus* Neum.	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Morning Market.
11th July	*R. oculatus* Neum.	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Morning Market.
12th July	*R. evertsi* Neum.	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Morning Market.
15th July	*H. aegyptium* Linn.	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Morning Market.
14th July	*H. aegyptium* Linn.	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Morning Market.
14th July	*R. oculatus* Neum.	—	Hare, *L. zuluensis*	Pretoria, Transvaal.
17th July	*R. oculatus* Neum. *H. aegyptium* Linn.	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Morning Market.
21st July	*R. oculatus* Neum.	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Morning Market.
26th July	*R. oculatus* Neum.	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Morning Market.
26th July	*R. oculatus* Neum.	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Morning Market.
28th July	*H. aegyptium* Linn.	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Morning Market.
29th July	*Ixodes rasus* Neum. *Boophilus*	—	Impala, *Aepyceros melampus* Lcht.	Pretoria, Transvaal.
31st July	*B. decoloratus* Koch	—	Impala, *Aepyceros melampus* Lcht.	Pretoria, Transvaal, Morning Market.
3rd August	*R. oculatus* Neum.	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Morning Market.
7th August	*R. appendiculatus* Neum. *B. decoloratus* Koch	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Morning Market.
8th August	*H. aegyptium* Linn.	—	Hare, *L. zuluensis*	Pretoria, Transvaal. Morning Market.
9th August	*R. oculatus* Neum.	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Morning Market.
31st July	*R. appendiculatus* Neum. *A. hebraeum* Koch	—	Sheep	Klasserie, Transvaal, Farm Scotia.
31st July	*A. hebraeum* Koch	—	Goat	Klasserie, Transvaal, Farm Scotia.
1st August	*A. hebraeum* Koch *H. leachii* Audouin	—	Dog	Klasserie, Transvaal, Farm Orinoco.
30th July	*H. aegyptium* Linn. *R. evertsi* *R. oculatus* Neum.	—	Hare, *L. zuluensis*	Klasserie, Transvaal, Farm Fleur-de-Lys.
30th July	*R. evertsi* Neum. *R. oculatus* Neum. *H. aegyptium* Linn.	—	Hare, *L. zuluensis*	Klasserie, Transvaal, Farm Fleur-de-Lys.
30th July	*R. oculatus* Neum. *Hyalomma* sp.	—	Hare, *L. zuluensis*	Klasserie, Transvaal, Farm Fleur-de-Lys.

SOUTH AFRICAN TICK RECORDS—(*continued*).

1928.	Ticks.	Parasites.	Hosts.	Localities.
30th July	*R. evertsi* Neum. *R. oculatus* Neum. *Hyalomma* sp.	—	Hare, *L. zuluensis*	Klasserie. Transvaal, Farm Scotia.
30th July	*R. appendiculatus* Neum.	—	Goat	Klasserie, Transvaal, Farm Scotia.
31st July	*H. aegyptium* Linn.	—	Hare, *L. zuluensis*	Klasserie, Transvaal, Farm Scotia.
30th July	*R. appendiculatus* Neum.	—	Goats	Klasserie, Transvaal, Farm Scotia.
31st July	*R. appendiculatus* Neum.	—	Calves	Klasserie, Transvaal, Farm Scotia.
31st July	*R. appendiculatus* Neum.	—	Calves	Klasserie, Transvaal, Farm Scotia.
31st July	*R. appendiculatus* Neum.	—	Calves	Klasserie, Transvaal, Farm Scotia.
30th July	*Rhipicephalus* (nymphs)	—	Hare, *L. zuluensis*	Klasserie, Transvaal, Farm Fleur-de-Lys.
1st August	*R. appendiculatus* Neum. *R. evertsi* Neum. *A. hebraeum* Koch	—	Hare, *L. zuluensis*	Klasserie, Transvaal, Farm Scotia.
1st August	*A. hebraeum* Koch *H. leachii* Audouin	—	Dog	Klasserie, Transvaal, Farm Orinoco.
21st July	*H. leachii* Audouin	—	Dog	Klasserie, Transvaal.
27th July	*R. appendiculatus* Neum. *A. variegatum* Fabr. *Ixodes* (nymphs) *A. hebraeum* Koch	—	Dog	Klasserie, Transvaal.
27th July	*Rhipicephalus* (nymphs)	—	Dogs	Klasserie, Transvaal.
27th July	*R. appendiculatus* Neum. *A. hebraeum* Koch *Ixodes pilosus* Koch	—	Dogs	Klasserie, Transvaal.
27th July	*R. evertsi* Neum. *H. aegyptium* Linn.	—	Hare, *L. zuluensis*	Klasserie, Transvaal.
28th July	*R. appendiculatus* Neum.	—	Dog	Sand River, Transvaal.
28th July	*A. hebraeum* Koch *H. leachii* Audouin	—	Dog	Klasserie, Transvaal.
27th July	*A. hebraeum* Koch	—	Dog	Klasserie, Transvaal.
28th July	*R. appendiculatus* Neum. *A. hebraeum* Koch	—	Dog	Klasserie, Transvaal.

26th July	*R. appendiculatus* Neum.	—	Yellow-footed squirrel, *Paraxerus cepapi* A. Smith	Klasserie, Transvaal, Farm Scotia.
27th July	*A. hebraeum* Koch	—	Dog	Klasserie, Transvaal.
	Ixodes sp. (nymphs)			
28th July	*R. appendiculatus* Neum.	—	Dog	Sand River, Transvaal.
27th July	*A. hebraeum* Koch	—	Dog	Klasserie, Transvaal.
6th August	*B. decoloratus* Koch	—	Sable antelope, *Ozanna nigra* Harris	Acorn Hoek, Transvaal, Spring Valley.
6th August	*H. leachii* Audouin	—	Dog	Acorn Hoek, Transvaal, Spring Valley.
7th August	*R. evertsi* Neum.	—	Goat	Acorn Hoek, Transvaal, Spring Valley.
	R. appendiculatus Neum.			
	A. variegatum Fabr.			
	A. hebraeum Koch			
	B. decoloratus Koch			
	Boophilus			
8th August	*R. appendiculatus* Neum.	—	Bull	Satara, Transvaal, Kruger Park.
	A. hebraeum Koch			
7th August	*A. hebraeum* Koch	—	Bull	Acorn Hoek, Transvaal, Spring Valley.
	B. decoloratus Koch			
	R. appendiculatus Neum.			
	H. aegyptium Linn.			
28th July	*R. oculatus* Neum.	—	No host given	Messina, Transvaal.
11th August	*R. oculatus* Neum.	—	Impala, *Aepyceros melampus* Lcht.	Moea Maru (?).
22nd August	*R. distinctus* Bedf.	—	Dassie, *Procavia coombsi* Rbts.	Onderstepoort, Stock Farm.
	H. cooleyi Bedf.			
	Rhipicentor bicornis			
29th August	*R. oculatus* Neum.	—	Hare, *L. ochropus* Wagn.	Bloemfontein, O.F.S.
	H. aegyptium Linn.			
28th August	*R. oculatus* Neum.	—	Hare, *L. ochropus* Wagn.	Glen, O.F.S.
	H. aegyptium Linn.			
29th August	*H. aegyptium* Linn.	*	Hare, *L. zuluensis*	Pretoria, Transvaal, Morning Market.
26th August	*R. evertsi* Neum.	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Pretorius Farm.
	H. aegyptium Linn.			
28th August	*R. evertsi* Neum.	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Pretorius Farm.
	Hyalomma sp.			
30th August	*H. aegyptium* Linn.	*	Hare, *L. zuluensis*	Pretoria, Transvaal, Pretorius Farm.
30th August	*H. aegyptium* Linn. (nymphs)	*	Hare, *L. zuluensis*	Pretoria, Transvaal, Nefdt Farm.

SOUTH AFRICAN TICK RECORDS—(*continued*).

1928.	Ticks.	Parasites.	Hosts.	Localities.
30th August	*R. evertsi* Neum.	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Nefdt Farm.
	H. aegyptium Linn.			
31st August	*R. evertsi* Neum.	*	Hare, *L. zuluensis*	Pretoria, Transvaal, Nefdt Farm.
31st August	*R. oculatus* Neum.	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Nefdt Farm.
30th August	*Rhipicephalus* (nymphs)	*	Hare, *L. zuluensis*	Pretoria, Transvaal, Nefdt Farm.
31st August	*R. evertsi* Neum.	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Nefdt Farm.
31st August	*R. evertsi* Neum.	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Nefdt Farm.
1st September	*H. aegyptium* Linn.	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Nefdt Farm.
31st August	*R. evertsi* Neum.	*	Hare, *L. zuluensis*	Pretoria, Transvaal, Nefdt Farm.
31st August	*R. evertsi* Neum.	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Nefdt Farm.
30th August	*Hyalomma* sp. (nymphs)	*	Hare, *L. zuluensis*	Pretoria, Transvaal, Nefdt Farm.
31st August	*Rhipicephalus* sp.	—	Hare, *L. capensis*	Grootfontein, O.F.S.
	R. oculatus Neum.			
30th August	*R. oculatus* Neum.	—	Hare, *L. capensis*	Middelburg, C.P.
31st August	*R. oculatus* Neum.	—	Hare, *L. capensis*	Grootfontein, O.F.S.
28th August	*H. leachii* Audouin	—	Dog	Grootfontein, O.F.S.
7th September	*H. aegyptium* Linn.	*	Hare, *L. zuluensis*	Pretoria, Transvaal, Nefdt Farm.
3rd September	*R. evertsi* Neum.	—	Cattle	Fort Beaufort, C.P.
5th September	*R. evertsi* Neum. (nymphs)	—	Cattle	Fort Beaufort, C.P.
6th September	*R. evertsi* Neum.	—	—	Fort Beaufort, C.P.
	R. appendiculatus Neum.			
3rd September	*R. evertsi* Neum.		Sheep	Fort Beaufort, C.P.
4th September	*R. evertsi* Neum.	—	Hares, *L. capensis*	Fort Beaufort, C.P.
	H. aegyptium Linn.	—		
13th September	*R. evertsi* Neum.		Calf	Hartebeestpoort Dam, near Pretoria, Transvaal, Smith's Farm.
	H. aegyptium Linn.	—		
	R. appendiculatus Neum.			
5th September	*Argas persicus* Oken		Fowls	Fort Beaufort, C.P.
5th September	*Boophilus*	—	Cattle	Fort Beaufort, C.P.
5th September	*R. evertsi* Neum.	—	Cattle	Fort Beaufort, C.P.
	A. hebraeum Koch	—		

12th September...	*R. oculatus* Neum. *R. evertsi* Neum. *R. appendiculatus* Neum.	—	Cattle	Bathurst, C.P.
12th September...	*R. appendiculatus* Neum. *R. evertsi* Neum. *A. hebraeum* Koch	—	Cattle	Bathurst, C.P.
12th September...	*R. evertsi* Neum. *R. appendiculatus* Neum.	—	Cattle	Bathurst, C.P.
12th September...	*R. appendiculatus* Neum. *Ixodes* sp. (nymphs)	—	Cattle	—
14th September...	*R. evertsi* Neum. *R. appendiculatus* Neum.	—	Cattle	Klemmond.
14th September...	*Boophilus*	—	Cattle	Klemmond.
21st September...	*H. aegyptium* Linn.	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Nefdt Farm.
18th September...	*Boophilus*	—	Cattle	Queenstown, C.P.
18th September...	*Boophilus*	—	Cattle	Queenstown, C.P.
18th September...	*R. evertsi* Neum.	—	Cattle	Queenstown, C.P.
18th September...	*R. evertsi* Neum.	—	Cattle	Queenstown, C.P.
18th September...	*R. evertsi* Neum.	—	Cattle	Queenstown, C.P.
18th September...	*R. evertsi* Neum.	—	Cattle	Queenstown, C.P.
22nd September..	*R. oculatus* Neum. *H. aegyptium* Linn.	—	African bulls	Bloemfontein, O.F.S.
27th September...	*R. evertsi* Neum. *B. decoloratus* Koch *H. aegyptium* Linn.	—	Cattle	Pretoria, Transvaal, Nefdt Farm.
27th September...	*H. aegyptium* Linn. *Boophilus*	—	Cattle	Pretoria, Transvaal, Nefdt Farm.
27th September...	*R. evertsi* Neum.	—	Cattle	Pretoria, Transvaal, Nefdt Farm.
1st October.....	*Ixodes* sp.	—	Hare, *L. zuluensis*	Wolfenada.
1st October.....	*R. oculatus* Neum.	*	Hare, *L. zuluensis*	Pienaar's River, Transvaal.
1st October.....	*R. oculatus* Neum.	—	Hare, *L. zuluensis*	Pienaar's River, Transvaal.
1st October.....	*R. oculatus* Neum.	—	Hare, *L. zuluensis*	Pienaar's River, Transvaal.
2nd October.....	*R. evertsi* Neum.	—	Impala, *Aepyceros melampus* Lcht.	Pienaar's River, Transvaal.
1st October.....	*R. oculatus* Neum.	—	Hare, *L. zuluensis*	Pienaar's River, Transvaal.
1st October.....	*H. numidiana* Neum.	—	Spring hare, *Pedetes caffer satinea* Wr.	Pienaar's River, Transvaal.
1st October.....	*H. aegyptium* (nymphs) Linn.	—	Hare, *L. zuluensis*	Pienaar's River, Transvaal.

SOUTH AFRICAN TICK RECORDS—(*continued*).

1928.	Ticks.	Parasites.	Hosts.	Localities.
30th September...	*H. aegyptium* Linn..................	—	Hare, *L. zuluensis*...............	Pienaar's River, Transvaal.
30th September...	*R. appendiculatus* Neum...........	*	Hare, *L. zuluensis*...............	Pienaar's River, Transvaal.
30th September...	*R. oculatus* Neum.................	—	Hare, *L. zuluensis*...............	Pienaar's River, Transvaal.
30th September...	*R. oculatus* Neum.................	*	Hare, *L. zuluensis*...............	Pienaar's River, Transvaal.
30th September...	*H. aegyptium* Linn..................	*	Hare, *L. zuluensis*...............	Pienaar's River, Transvaal.
1st October.....	*H. aegyptium* Linn..................	—	Hare, *L. zuluensis*...............	Pienaar's River, Transvaal.
1st October.....	*H. leachii* Audouin...............	—	Spotted gennet, *Genetta ludia* Thos. & Schw.	Pienaar's River, Transvaal.
12th October.....	*R. sanguineus* Latr...............	—	Dog...........................	Onderstepoort, Transvaal.
12th October.....	*B. decoloratus* Koch..............	—	Cattle.........................	Pretoria, Transvaal, Nefdt Farm.
7th August......	*R. evertsi* Neum..................	—	Bull...........................	Acorn Hoek, Transvaal, Spring Valley.
30th August......	*R. oculatus* Neum.................	—	Hare, *L. zuluensis*...............	Pretoria, Transvaal, Nefdt Farm.
22nd September..	*R. oculatus* Neum.................	—	Hare, *L. ochropus* Wagn.........	Glen, O.F.S.
1st October.....	*R. oculatus* Neum.................	—	Hare, *L. zuluensis*...............	Pienaar's River, Transvaal.
25th September...	*R. evertsi* Neum..................	—	Cattle.........................	Middelburg, C.P.
1st October.....	*R. oculatus* Neum.................	—	Hare, *L. zuluensis*...............	Pienaar's River, Transvaal.
28th July........	*R. sanguineus* Latr...............	—	Dog...........................	Klasserie, Transvaal.
	A. variegatum Fabr.			
1st October.....	*R. oculatus* Neum.................	—	Hare, *L. zuluensis*...............	Pienaar's River, Transvaal.
3rd September...	*R. evertsi* Neum..................	—	Sheep..........................	—
20th September...	*R. evertsi* Neum..................	—	Cattle.........................	Middelburg, C.P.
9th August......	*R. evertsi* Neum..................	—	Duiker, *Sylvicapra grimmi* L....	Acorn Hoek, Transvaal, Spring Valley.
18th September...	*R. evertsi* Neum..................	—	Cattle.........................	Queenstown, C.P.
	H. aegyptium Linn.			
7th October.....	*R. evertsi* Neum..................	—	Cattle.........................	Pretoria, Transvaal, Nefdt Farm.
	B. decoloratus Koch			
	A. variegatum Fabr.			
28th August......	*H. leachii* Audouin...............	—	Meerkat........................	Ventersburg, O.F.S.
7th August......	R. *evertsi* Neum..................	—	Goat...........................	Acorn Hoek, Transvaal.
7th October.....	*R. evertsi* Neum..................	—	Cattle.........................	Nefdt Farm, Pretoria, Transvaal.
1st October.....	*R. oculatus* Neum.................	—	Hare, *L. zuluensis*...............	Pienaar's River, Transvaal.
28th September...	*R. sulcatus* Neum.................	—	*Pronolagus randensus*...........	Pretoria, Transvaal, Silverton Farm.
7th August......	*R. sanguineus* Latr...............	—	Hare, *L. zuluensis*...............	Acorn Hoek, Transvaal, Spring Valley.

2nd October	*R. evertsi* Neum.	—	Sheep	Wintershoek, Rounderbult.
1st October	*R. oculatus* Neum.	—	Hare, *L. zuluensis*	Wolfenada.
13th September	*R. simus* Koch	—	Cattle	—
	R. oculatus Neum.			
17th October	*R. evertsi* Neum.	—	Horse	—
30th July	*R. oculatus* Neum.	—	Hare, *L. zuluensis*	Acorn Hoek, Transvaal, Fleur-de-Lys Farm.
12th September	*R. simus* Koch	—	Cattle	—
	B. decoloratus Koch			
	H. silacea Rob.			
	H. vriegatum Fabr.			
	R. evertsi Neum.			
22nd September	*R. evertsi* Neum.	—	Africander bull	Middleburg.
14th September	*R. evertsi* Neum.	—	Cattle	Klemmond.
	B. decoloratus Koch			
5th September	*R. oculatus* Neum.	—	Ox	Fort Beaufort, C.P.
1st October	*R. oculatus* Neum.	—	Hare, *L. zuluensis*	Pienaar's River, Transvaal.
2nd September	*R. oculatus* Neum.	—	Africander bulls	Bloemfontein, O.F.S.
5th September	*R. simus* Koch	—	—	Fort Beaufort.
30th July	*R. evertsi* Neum.	—	Steenbok, *Raphiceros campestris*	Klasserie, Transvaal.
1st October	*R. oculatus* Neum.	—	Hare, *L. zuluensis*	Pienaar's River, Transvaal.
25th July	*R. sanguineus* Latr.	—	Dog	Klasserie, Transvaal, Scotia Farm.
31st July	*R. evertsi* Neum.	—	Sheep	Klasserie, Transvaal, Scotia Farm.
1st September	*H. aegyptium* Linn.	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Nefdt Farm.
	R. evertsi Neum.			
31st August	*R. evertsi* Neum.	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Nefdt Farm.
27th July	*R. simus* Koch	—	Dog	Klasserie, Transvaal.
	R. sanguineus Latr.			
18th September	*R. evertsi* Neum.	—	Cattle	Queenstown, C.P.
31st July	*R. evertsi* Neum.	—	Sheep	Klasserie, Transvaal, Scotia Farm.
	A. hebraeum Koch			
31st July	*R. evertsi* Neum.	—	Goat	Klasserie, Transvaal, Scotia Farm.
1st October	*H. aegyptium* Linn.	—	Off the ground	Wolfenada.
31st July	*H. aegyptium* Linn.	—	Horse	Klasserie, Transvaal, Scotia Farm.
13th September	*B. decoloratus* Koch	—	Cattle	—
7th August	*B. decoloratus* Koch	—	Bull	Acorn Hoek, Transvaal, Spring Valley.
4th August	*R. evertsi* Neum.	—	Sable antelope, *Ozanna nigra* Harris	Acorn Hoek, Transvaal, Spring Valley.
	B. decoloratus Koch			

SOUTH AFRICAN TICK RECORDS—(*continued*).

1928.	Ticks.	Parasites.	Hosts.	Localities.
6th July	*B. decoloratus* Koch	—	Hare, *L. zuluensis*	Messina, Transvaal, Dongola Farm.
8th August	*B. australis* Fuller	—	Bull	Satara, Transvaal, Kruger Park.
12th October	*B. decoloratus* Koch	—	Cattle	Pretoria, Transvaal, Nefdt Farm.
14th September	*B. decoloratus* Koch	—	Cattle	Bathurst, C.P.
	R. evertsi Neum.			
	H. silacea Rob.			
6th August	*B. decoloratus* Koch	—	Sable antelope, *Ozanna nigra* Harris	Acorn Hoek, Transvaal, Spring Valley.
12th October	*B. decoloratus* Koch	—	Cattle	Pretoria, Transvaal, Nefdt Farm.
27th September	*B. decoloratus* Koch	—	Cow	Pretoria, Transvaal, Nefdt Farm.
12th September	*Boophilus*	—	Calf	—
20th September	*B. decoloratus* Koch	—	Cattle	Middelburg, C.P.
5th September	*H. aegyptium* Linn.	—	Cattle	Fort Beaufort, C.P.
12th October	*H. aegyptium* Linn.	—	Cattle	Pretoria, Transvaal, Nefdt Farm.
20th September	*H. aegyptium* Linn.	—	Cattle	Middelburg, C.P.
31st July	*H. aegyptium* Linn.	—	Hog	Klasserie, Transvaal, Scotia Farm.
30th August	*Rhipicephalus* sp. (nymphs)	—	Calf	Pretoria, Transvaal, Nefdt Farm.
7th October	*H. aegyptium* Linn.	—	Cattle	Pretoria, Transvaal, Nefdt Farm.
22nd September	*H. aegyptium* Linn.	—	Africander bull	Winburg District, O.F.S.
12th October	*H. aegyptium* Linn.	—	Cattle	Pretoria, Transvaal, Nefdt Farm.
	A. hebraeum Koch			
31st August	*Hyalomma* (nymphs)	—	Hare, *L. zuluensis*	Pretoria, Transvaal, Nefdt Farm.
20th September	*H. aegyptium* Linn.	—	Cattle	Middelburg, C.P.
4th August	*H. aegyptium* Linn.	—	Sable antelope, *Ozanna nigra* Harris	Acorn Hoek, Transvaal, Spring Valley.
	B. decoloratus Koch			
25th July	*H. aegyptium* Linn.	—	Horse	Klasserie, Transvaal, Scotia Farm.
18th September	*H. aegyptium* Linn.	—	Cattle	Queenstown, C.P.
17th September	*Argas persicus* Oken	—	—	Queenstown, C.P.
17th September	*Argas persicus* Fis.	—	Pettertrees	Queenstown, C.P.
15th September	*Argas persicus* Fis.	—	Domestic fowl	Fort Beaufort, C.P.
18th September	*Ornithodoros megnini* Dugès	—	Cattle	Queenstown, C.P.
9th September	*A. hebraeum* Koch	—	Cattle	—
20th September	*A. hebraeum* Koch	—	Cattle	Middelburg, C.P.
	R. evertsi Neum.			

27th July........	*A. hebraeum* Koch (nymphs)........	—	Dog..........................	Klasserie, Transvaal.
7th August......	*A. hebraeum* Koch...............	—	Goat.........................	Acorn Hoek, Transvaal, Spring Valley.
20th September...	*A. hebraeum* Koch...............	—	Cattle.......................	Middelburg, C.P.
27th September...	*A. hebraeum* Koch...............	—	Cow..........................	Pretoria, Transvaal, Nefdt Farm.
25th July........	*A. hebraeum* Koch...............	—	Horse........................	Klasserie, Transvaal, Scotia Farm.
9th September...	*A. hebraeum* Koch...............	—	Cattle.......................	Bathurst, C.P.
7th August......	*A. hebraeum* Koch...............	—	Bull.........................	Acorn Hoek, Transvaal, Spring Valley.
	R. evertsi Neum.			
12th October.....	*H. aegyptium* Linn...............	—	Cattle.......................	Pretoria, Transvaal, Nefdt Farm.
	R. evertsi Neum.			
5th September...	*R. evertsi* Neum.................	—	Cattle.......................	Fort Beaufort, C.P.
7th October.....	*A. hebraeum* Koch...............	—	Cattle.......................	Pretoria, Transvaal, Nefdt Farm.
12th October.....	*A. hebraeum* Koch...............	—	Cattle.......................	Pretoria, Transvaal, Nefdt Farm.
31st July........	*A. hebraeum* Koch...............	—	Pig..........................	Klasserie, Transvaal, Scotia Farm.
31st July........	*A. hebraeum* Koch...............	—	Sheep........................	Klasserie, Transvaal, Scotia Farm.
25th July........	*A. hebraeum* Koch...............	—	Dog..........................	Acorn Hoek, Transvaal, Spring Valley.
7th October.....	*A. hebraeum* Koch...............	—	Cattle.......................	Pretoria, Transvaal Nefdt Farm.
	R. evertsi Neum.			
5th September...	*A. hebraeum* Koch...............	—	Cattle.......................	Fort Beaufort, C.P.
8th August......	*A. hebraeum* Koch...............	—	Bull.........................	Satara, Transvaal, Kruger Park.
8th August......	*A. hebraeum* Koch...............	—	Horse........................	Satara, Transvaal, Kruger Park.
	R. evertsi Neum.			
31st July........	*A. hebraeum* Koch...............	—	Goat.........................	Klasserie, Transvaal, Scotia Farm.
	R. evertsi Neum.			
28th July........	*A. hebraeum* Koch...............	—	Dog..........................	Klasserie, Transvaal.
27th July........	*A. hebraeum* Koch...............	—	Dog..........................	Klasserie, Transvaal.
19th July........	*A. vespertilionis* Latr.............	—	In a house...................	Barberton, Transvaal.
6th September...	*R. evertsi* Neum.................	—	No host given................	Fort Beaufort, C.P.
10th October.....	*R. evertsi* Neum.................	—	Sheep, horses................	Harding, Natal.
28th July........	*R. appendiculatus* Neum...........	—	Dog..........................	Klasserie, Transvaal.
27th July........	*R. appendiculatus* Neum..........	—	Dog..........................	Klasserie, Transvaal.
10th August......	*O. moubata* Mur..................	—	Kaffir hut...................	Acorn Hoek, Transvaal.
18th September...	*O. megnini* A. Dugès..............	—	Cattle.......................	Queenstown, C.P.
18th September...	*O. megnini* A. Dugès..............	—	Cattle.......................	Queenstown, C.P.
27th September...	*O. megnini* A. Dugès..............	—	Horses.......................	Petrusburg, O.F.S.
18th September...	*O. megnini* A. Dugès..............	—	Cattle.......................	Queenstown, C.P.
15th September...	*O. megnini* A. Dugès..............	—	Cattle.......................	Queenstown, C.P.

SOUTH AFRICAN TICK RECORDS—(*continued*).

1928.	Ticks.	Parasites.	Hosts.	Localities.
27th July	*H. leachii* Audouin	—	Dog	Klasserie, Transvaal.
8th August	*H. leachii* Audouin	—	Leopard, *Panthera suahelica* Neum.	Satara, Transvaal, Kruger Park.
7th August	*H. leachii* Audouin	—	Lion, *Leo leo krugeri* Rbts.	Acorn Hoek, Transvaal, Blyde River.
18th September	*H. leachii* Audouin	—	Mongoose	Pretoria, Transvaal.
27th July	*H. leachii* Audouin	—	Dog	Klasserie, Transvaal.
28th July	*H. leachii* Audouin	—	Dog	Klasserie, Transvaal.
27th July	*H. leachii* Audouin	—	Dog	Klasserie, Transvaal.
12th September	*R. appendiculatus* Neum.	—	Cattle	Bathurst, C.P.
20th July	*H. leachii* Audouin	—	Hare, *L. zuluensis*	Zululand.
2nd August	*H. leachii* Audouin	—	Dog	Klasserie, Transvaal, Orinocco.
27th July	*Ixodes pilosus* Koch	—	Dog	Klasserie, Transvaal.
5th September	*Ixodes pilosus* Koch	—	No host given	Fort Beaufort, C.P.
15th July	*Aponomma* sp. (larvae)	—	Mamba snake, *Dendraspis angusticeps* Sm.	Barberton, Transvaal.

TICK PARASITES FOUND.

Of the two hundred and sixty lots of ticks collected, thirteen were parasitized with *H. hookeri*. In all cases parasites were found only in nymphal ticks, in fact in all countries, and with all ticks that have been attacked by this parasite, the nymphal stage only is parasitized, so far as is known. It is shown also that the following tick species were parasitized:—

Hyalomma aegyptium, six lots.
Rhipicephalus oculatus, two lots.
Rhipicephalus evertsi, two lots.
Rhipicephalus appendiculatus, one lot.
Rhipicephalus sp., one lot.
Hyalomma sp., one lot.

Further examination of this tabulated information shows that in every case the parasitized ticks were feeding on the hare, *Lepus zuluensis*.

The parasitized ticks were found only in the Transvaal. They were found on the farms of Andries W. J. Pretorius and T. S. Nefdt, on the shore of the Hartebeestpoort Dam, and on the stock farm of Pretorius at Pienaar's River known as "Bushfeld" farm. No attempt was made to determine the limits of the areas where the parasites were present. The dates of the recovery of parasites were scattered from 9th June to 30th September. Bearing in mind that the collecting period was from 4th June to 17th October and that few ticks were taken after 1st October, it is seen that the records indicate that parasites were present through most of the colder months, and suggests that they would likely be even more active during the warmer part of the year.

Further comments on the biology of this tick parasite will be of some interest. A very considerable portion of the ticks recorded in the table were adults, but nearly all those taken from rabbits and other small animals were nymphs or larvae. It has been pointed out on an earlier page, that in collecting nymphs and larvae we cannot expect them to develop recognizable parasitism, unless they are at least two-thirds engorged. It is quite probable, therefore, that more parasitism was present than is shown. In similar work done by the writer in America, it was found that if the animals are taken alive by trapping and held in cages enclosed in bags, all of the ticks can be recovered fully fed. The trap method is of particular value in recovering parasitized ticks when checking on the effectiveness of attempted colonization of parasites in nature.

The writer attaches no particular significance to the taking of *H. hookeri* in ticks found only on hares, for it was taken on deer in France, by Brumpt, and has been found in ticks from dogs several times, as well as from ticks on other host animals. It appears to be true that this insect will attack larval or nymphal ticks on either large or small animals. In the South African material here concerned, the immature ticks of the parasitized species listed above were not often found on the larger animals.

H. hookeri has been taken from a wide variety of ticks. It has been recorded elsewhere in the literature as parasitizing the following: *Haemaphysalis leporis-palustris*, *R. evertsi*, *R. sanguineus*, *Ixodes ricinus* and *Dermacentor parumapertus marginatus*. It has been recovered in nature in America in *D. andersoni* and *Dermacentor variabilis*, in areas where parasites have been liberated in attempted biological control. In the tabulation here presented are added three species, and two instances in *Rhipicephalus* and *Hyalomma* when the species was not determined. It is apparent, therefore, that this parasite attacks species of five genera of ticks which show some range of habits. We do not know of any case in which it has attacked a tick which remains on one host for feeding in its three stages. It would be of particular interest to attempt to establish this insect as a parasite of *Boophilus decoloratus*. In the case of this tick the parasites would have opportunity to lay eggs in both larvae and in nymphs, both while unfed and while feeding, and the ticks might remain attached to the host animal while the parasites were maturing, thus insuring that the adult parasites would be in the presence of ticks on the host when they emerge.

SPECIALIZATION IN *HUNTERELLUS HOOKERI*.

In an evolutionary sense we must recognize that this tick parasite is highly specialized. It is a parasite of ticks only, so far as we know. It would be difficult to imagine any way in which these insects could become injurious to man's interests unless it were to become a secondary parasite with another species of tick parasite as primary, in which case it might tend to destroy the primary by feeding on it within the host tick. We know of no such instance. Numerous points, both morphological and biological, could be cited to show that there is specialization in this insect's attack on ticks, but apparently this specialization has not gone far enough to make these insects of particular value in the control of any ticks or type of tick that we know of, unless possibly in the case of *B. decoloratus* and other species of the one host type.

A parasite to be of maximum value should be characterized by a high degree of host specificity. It is perhaps not going too far to say that we can imagine a type and degree of specialization that would characterize a parasite of such special value. One example, though hypothetical, may serve to show what it is intended to mean. There might be found some parasite with these or similar further specialized points, namely, ability to recognize as food only one species of tick, or a very few species, and able by especially developed senses to locate these ticks, or their haunts. Such a parasite might be of particular value. The writer does not intend to say that *H. hookeri* cannot be of considerable value in the control of such ticks as *H. aegyptium*, *R. oculatus*, and *R. evertsi*, in the climate of South Africa.

PERCENTAGE OF PARASITIZED TICKS.

It is of interest to note that a large proportion, or about 80 per cent. of the nymphs collected near Hartebeestpoort Dam, and at the location on Pienaar's River, were parasitized. In figuring this percentage only those living nymphs which had been sufficiently fed

to produce parasites were counted. It is possible that a higher percentage of parasitism might have been found if all of the nymphs had been fully fed or if latent parasitism had been taken into account.

ADULT PARASITES FOUND IN FUR OF RABBITS.

Previous to the writer's visit to Transvaal, in all of the published records of the finding of *H. hookeri*, the insects had been recovered only in immature stages in nymphs of the several species. It was of interest therefore that, while working on the Nefdt farm on 7th September, a rabbit was shot at about 11 o'clock a.m., and in examining it, eight adult parasites, all females, were found in the fur of the animal. It was a bright, hot day and the estimated temperature was 90° F. There had always been the question as to whether the female parasites lay eggs in ticks, on the ground, in nests of the host animals, or in the ticks while they are feeding, or about to feed, on the host. This observation appears to show that the parasite eggs are laid on the animals, although we still do not know whether they also laid eggs in the ticks on the ground, or in the nests.

There has since been published by Doctor C. B. Philip (25) an account of the discovery of *H. hookeri* in the hair of dogs at Lagos, Nigeria. The same author also states that at least 90 per cent. of the ticks (*Rhipicephalus sanguineus*) were parasitized, but the ticks were still very abundant. In this connection it would be of interest if we might know if the parasites had been recently introduced at Lagos, and further to know if the parasites were approaching one of the probable periods of maximum abundance.

A POSSIBLE NEW PARASITE SEEN.

While stationed on the Nefdt farm, one living specimen of an insect was seen that may have been a parasite of ticks. Knowledge of the incident may be of some interest to future workers.

In the morning of 1st September, there were for examination six rabbits which had been shot late in the previous evening. When shot, they were dropped into two collecting bags of cloth, four in one bag and two in another, and the ends of the bags were tied. In examining the last rabbit, one of the two in one bag, a living insect was seen on one of the rabbits. When the ear was opened with the fingers, the insect ran to the margin of the ear and flew away. It would have been interesting to capture the insect and put it in a vial, but the writer had not over a second or two in which to examine it. It was clearly a hymenopterous insect and it was thought to belong to the Chalcididae or Proctotrupidae. The thorax was relatively large, showing strong muscular development and the hind femora were thickened. Thinking that this insect might be recovered again, an experiment was made with caged animals placed out in the immediate vicinity where the insect was seen. Wire cages, laboratory rabbits, and nymphal ticks (*Rhipicephalus appendiculatus*) were obtained through the courtesy of the laboratory at Onderstepoort. The cages, six in number, containing rabbits and ticks, were placed in the field, hoping that some of the insects would reach the ticks through the mesh of the cages. They were left out as long as possible,

being brought in just before the ticks had completed feeding. The ticks were recovered and held under observation. No parasites developed. If the procedure had included the finding of the adult ticks that emerged from the fed nymphs, it is just possible that we might have discovered parasitism due to latency.

SUMMARY OF THE PRESENT STATUS OF OUR KNOWLEDGE OF THE FAMILY *ENCRYTIDAE*.

There have been described three species of tick parasites all closely related and classified in the *Encrytidae*. They are: *Ixodiphagus texanus* Howard (17), *Hunterellus hookeri* Howard (18), *Ixodiphagus caucurtei* du Buysson (16). Dr. A. B. Gahan and Dr. C. F. W. Mueseback, of the U.S. National Museum, have recently concluded and will soon publish that *H. hookeri* and *I. caucurtei* are one and the same insects, and the name *H. hookeri* will be retained as having priority. *I. texanus*, described in 1907, was not seen again until taken by Mr. Carl Larson and Mr. Roger Cooley, two assistants in the U.S. Public Health Service, working in the field near Mayfield, Idaho (U.S.A.). Two parasitized nymphs were found in a considerable number of ticks, *Ixodes hexagonus* var. *cookei* Packard, taken on a woodchuck, on 28th June, 1932. Several generations were reared at the laboratory, and while we know that it is very similar in habits to *H. hookeri*, we lack sufficient information to be able to express a view on the possible value of the insect in biological control.

I. texanus has been found only in the United States, but *H. hookeri* has been found in France, India, Indo-China, Cuba, Brazil, Union of South Africa, Nigeria, South West Africa, and the United States.

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Without the generous assistance, both official and personal, given me by the people of South Africa, it would have been difficult or impossible to accomplish the survey that was made. On behalf of the Montana State Board of Entomology, and personally, I desire to express sincere appreciation to the Union of South Africa, through the following:—

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NOTE.—There are also now in manuscript the following:—

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 3, Number 1, July, 1934.

Results of a Mosquito Survey at Onderstepoort during the Summer 1931-32 in connection with the Transmission of Horsesickness.

By

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Notwithstanding the fact that work on the transmission of horsesickness was commenced as long as 30 years ago, the actual natural transmission of this disease has up to the present not been cleared up.

According to general opinion mosquitoes are strongly suspected of being the transmitting agents, although this viewpoint does not find unanimous support amongst the farming community in South Africa amongst whom a diversity of opinions exists. We will, however, discuss these latter theories more fully in another paper confining ourselves here solely to the main facts.

Horsesickness is not contagious. Its occurrence under natural conditions is restricted normally to the summer months and the severity of an outbreak depends mainly on the amount of rainfall. Infections generally take place only at night, round about sunset or sunrise. Horses kept in screened stables at night escape infection even in known bad horsesickness areas. These facts collectively are to be regarded as strong indications that mosquitoes are involved in the transmission of this disease and when we started our work on the transmission of horsesickness in 1931 mosquitoes received primary attention.*

* This paper constitutes the first of a series dealing with the transmission of horsesickness and bluetongue of sheep, carried out at the Onderstepoort Laboratories.

One of the authors (O.N.) remained in South Africa during the winter 1931 and the summer 1931/32 as a foreign Fellow of the Rockefeller Foundation and continued work during the summer 1933, assisted by a Research Grant of the Rockefeller Foundation and a grant from the South African Government. He wishes to express his deep indebtedness to the Rockefeller Foundation for the generous support granted him, which made it possible for him to study these interesting problems, and to Dr. P. J. du Toit, Director of Veterinary Services and Animal Industry, for the generous hospitality extended to him at the Onderstepoort Laboratories. He also wishes to express his gratitude to Prof. Dr. L. de Blieck, Director of the Institute for Parasitic and Infectious Diseases of Utrecht, who allowed him the long leave, necessary for the journeys to South Africa.

In order to obtain the necessary information about the species of mosquitoes most probably connected with the transmission of the disease under review, a mosquito survey was carried out at Onderstepoort during the summer months of 1931-32. At the same time. however, suitable experimental methods had to be worked out and the transmission experiments themselves conducted. The desired degree of completeness in this survey could, therefore, not be expected.

The original farm on which Onderstepoort stands was known in former years as a particularly bad place for horsesickness. Although subsequently many important alterations have taken place in the neighbourhood of Onderstepoort, notably the construction of the Bon Accord Dam, the site was regarded as suitable for this research, as we considered that the breeding places of the most probable transmitters could quite reasonably still be expected to exist. The success of the work, however, depended largely on climatic factors, namely, as to whether these would be such as to result in a real horsesickness season or not. In this respect we were disappointed.

I. CLIMATIC CONDITIONS DURING THE SEASON 1931-32 AT ONDERSTEPOORT.

As previously stated, all available information tends to indicate that outbreaks of horsesickness depend mainly on the amount of rainfall during the summer months. The rainfall recorded from October, 1931, until April, 1932, at the meteorological post at Onderstepoort is shown in Table 1.

TABLE 1.

	1931.			1932.			
	October.	November.	December.	January.	February.	March.	April.
	Inches.	Inches.	Inches.	Inches.	Inches.	Inches.	Inches.
1	—	—	·14	—	—	—	—
2	—	·13	·01	—	·66	—	—
3	—	·025	—	·40	·10	—	—
4	·70	·28	·44	—	1·05	—	—
5	—	—	—	—	·30	—	—
6	—	1·55	—	·29	—	—	—
7	—	·11	—	—	—	—	—
8	—	·12	—	—	—	·07	—
9	—	·01	—	—	—	1·58	—
10	·10	·53	—	—	—	—	—
11	·01	—	—	·03	—	—	—
12	—	—	—	—	—	·05	—
13	—	—	—	—	—	—	—
14	—	—	—	—	·16	—	—
15	—	—	—	—	—	—	—
16	—	—	—	—	—	—	—
17	—	—	·40	—	1·40	—	—
18	—	—	·12	—	—	—	—
19	—	·02	—	·04	·43	—	—
20	·39	—	·05	1·30	·05	·29	—
21	·46	·09	·14	1·77	—	·05	—
22	—	·65	·01	—	—	—	—
23	·09	·64	—	—	—	—	·17
24	—	—	—	—	—	—	·23
25	—	·24	—	—	·20	—	·04
26	—	·30	·07	·02	—	—	·31
27	·03	—	—	—	—	—	—
28	·22	—	—	·14	·48	—	—
29	·45	—	—	—	·02	·04	—
30	—	·38	—	—	—	—	—
31	—	—	·27	—	—	—	—
Per Month..	2·45	5·075	1·65	3·99	4·85	2·08	·75

The total amount of rain during this period was 20·84 inches, the maximum per month 5·07 inches in November, and the minimum 0·75 inches in April. There were 9 days with rain in October, 15 days in November, 10 days in December, 8 days in January, 11 days in February, 6 days in March and 4 days in April, or 63 days altogether in 7 months. Only those days on which at least ¼ inch rain is recorded are of any real value, the soil immediately drying up again with less than this amount. In the season in question, therefore, we were confronted with 3 suitable days in October, 7 days in November, 3 days in December, 3 days in January, 4 days in February, 2 days in March and 1 day in April, giving a total of 23 days which were in any degree suitable.

To be sure of good breeding, at least one inch of rain is necessary to start with, which must be followed by an additional fall during the ensuing 5-7 days in order to ensure complete larval and pupal development. A rainfall of 1 inch or more, with 1·77 as the

maximum, was noted on 1 day in November, not at all in December, twice in January, twice in February, and once in March, altogether on 6 out of more than 200 days.

The rainfall actually corresponded with that of a very dry season which was further borne out by observations on plant life and the level of water present in the Bon Accord Dam, lying in the immediate neighbourhood of the Onderstepoort Laboratories, which was far below that of normal seasons. As a result of the dry season, only a few isolated cases of horsesickness occurred at Onderstepoort and the adjoining farms towards the end of the season, and in only one of these cases did no history of a previous immunization exist.

To obtain a clear idea of the rainfall in a bad horsesickness season, we must compare the rainfall in the season under review with that of the summer 1917-18 and 1922-23, when severe outbreaks of horsesickness occurred at Onderstepoort and the adjoining farm Kaalplaas, which also belongs to the Laboratory. This difference is clearly shown in Table 2 and Graph 1.

Graph I.

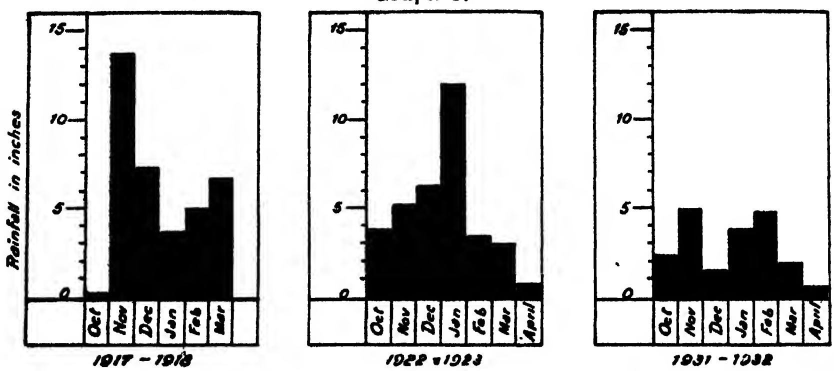

Fig. 1.—Monthly distribution of rain during two good horsesickness seasons (1917-1918 and 1922-1923) and during the season 1931-1932.

TABLE 2.

	1917-1918.	1922-1923.	1931-1932.
July	·40	—	1·43
August	2·07	3·07	—
September	·34	·31	—
October	·35	3·97	2·45
November	13·82	5·26	5·075
December	7·35	6·30	1·65
January	3·74	12·05	3·99
February	5·11	3·58	4·85
March	6·89	3·12	2·08
April	—	·96	·75
May	—	·01	—
June	—	·12	—
JULY–JUNE	40·07	38·75	22·275
NOVEMBER–APRIL	36·91	31·47	18·395

Comparison between the rainfall in 1917-1918 and 1922-1923, two notable horsesickness seasons, with the season under review.

2. SPECIES OF MOSQUITOES RECORDED FROM SOUTH AFRICA.

In South Africa the following 102 species of mosquitoes belonging to 13 different genera have been found up to the present: —

Tribe Anophelini.

1. *Anopheles (Anopheles) mauritianus* Daruty & d'Emmerez.
2. *Anopheles (Myzomyia) ardensis* (Theobald).
3. *Anopheles (Myzomyia) cinereus* Giles.
4. *Anopheles (Myzomyia) cinereus* Giles.
5. *Anopheles (Myzomyia) funestus* Giles.
   Anopheles (Myzomyia) funestus leesoni Evans.
6. *Anopheles (Myzomyia) gambiae* Giles.
7. *Anopheles (Myzomyia) jacobi* (Hill & Haydon).
8. *Anopheles (Myzomyia) listeri* De Meillon.
9. *Anopheles (Myzomyia) longipalpis* Theobald.
10. *Anopheles (Myzomyia) maculipalpis* Giles.
11. *Anopheles (Myzomyia) marshalli* (Theobald).
12. *Anopheles (Myzomyia) natalensis* (Hill & Haydon).
13. *Anopheles (Myzomyia) nili* Theobald.
14. *Anopheles (Myzomyia) pharoensis* Theobald.
15. *Anopheles (Myzomyia) pretoriensis* Theobald.
16. *Anopheles (Myzomyia) rhodesiensis* Theobald.
17. *Anopheles (Myzomyia) rufipes* (Gough).
18. *Anopheles (Myzomyia) squamosus* (Theobald).
19. *Anopheles (Myzomyia) squamosus sydippis* de Meillon.
20. *Anopheles (Myzomyia) theileri* Edwards.
19. *Anopheles (Myzomyi) squamosus cydippis* De Meillon.
20. *Anopheles (Myzomyia) demeilloni* Evans.
   Anopheles (Myzomyia) demeilloni carteri Evans & De Meillon.
20a. *Anopheles (Myzomyia) garnhami* Edwards.
   Anopheles (Myzomyia) garnhami walshi Evans and De Meillon.

Tribe Culicini.

21. *Aëdes (Aedimorphus) abnormalis* (Theobald).
22. *Aëdes (Rëdimorphus) albocephalus* (Theobald).
23. *Aëdes (Aëdimorphus) alboventralis* (Theobald).
24. *Aëdes (Aëdimorphus) apicoannulatus* (Edwards)
25. *Aëdes (Aëdimorphus) argenteopunctatus* (Theobald).
26. *Aëdes (Aëdimorphus) bevisi* Edwards.
27. *Aëdes (Aëdimorphus) capensis* (Edwards).
28. *Aëles (Aëdimorphus) cumminsi* (Theobald).
29. *Aëdes (Aëdimorphus) dentatus* (Theobald).
30. *Aëdes (Aëdimorphus) durbanensis* (Theobald).
31. *Aëdes (Aëdimorphus) fascipalpis* (Edwards).
32. *Aëdes (Aëdimorphus) filicis* Ingram & De Meillon.
33. *Aëdes (Aëdimorphus) furcifer* (Edwards).
34. *Aëdes (Aëdimorphus) marshalli* (Theobald).
36. *Aëdes (Aëdimorphus) minutus* (Theobald).
37. *Aëdes (Aëdimorphus) ochraceus* (Theobald).
39. *Aëdes (Aëdimorphus)punctothoracis* (Theobald).
40. *Aëdes (Aëdimorphus) quasiunivittatus* (Theobald).

41. *Aëdes (Aëdimorphus) tarsalis* (Newstead).
42. *Aëdes (Banksinella) lineatopennis* Ludlow.
Aëdes (Banksinella) lineatopennis circumluteola Theobald.
43. *Aëdes (Banksinella) luteolateralis* Theobald.
Aëdes (Banksinella) luteolateralis fiavinervis Edwards.
44. *Aëdes (Finlaya) barnardi* Edwards.
45. *Aëdes (Ochlerotatus) caballus* (Theoabld).
46. *Aëles (Stegomyia) argenteus* (Poiret).
47. *Aëdes (Stegomyia) metallicus* Edwards.
48. *Aëdes (Stegomyia) poweri* Theobald.
49. *Aëdes (Stegomyia)pseudonigeria* (Theobald).
50. *Aëdes (Stegomyia) unilineatus* (Theobald).
51. *Aëdes (Stegomyia) simpsoni* (Theobald).
52. *Aëdes (Stegomyia) subargenteus* Edwards.
53. *Aëdes (Stegomyia) vittatus* Bigot.
54. *Armigeres argenteoventralis* Theobald.
55. *Culex annulioris* Theobald.
56. *Culex aurantapex* Edwards.
57. *Culex bitaeniorhynchus* Giles.
58. *Culex consimilis* Newstead.
59. *Culex decens* Theobald.
60. *Culex duttoni* Theobald.
61. *Culex fatigans* Wiedemann.
62. *Culex laurenti* Newstead.
63. *Culex pallidocephalus* Theobald.
64. *Culex pérenguеyi* Edwards.
65. *Culex pipiens* Linné.
66. *Culex pulchrithorax* Edwards.
67. *Culex quasigelidus* Theobald.
68. *Culex rima* Theobald.
69. *Culex salisburiensis* Theobald.
70. *Culex sergenti* Theobald.
71. *Culex simpsoni* Theobald.
72. *Culex thalassius* Theobald.
73. *Culex theileri* Theobald.
74. *Culex trifilatus* Edwards.
75. *Culex univittatus* Theobald.
76. *Culex vansomeri* Edwards.
77. *Culex (Culicomyia) nebulosus* Theobald.
78. *Culex (Micraedes) inconspicuosus* Theobald.
79. *Eretmopodites chrysogaster* Graham.
80. *Eretmopodites quinquevittatus* Theobald.
81. *Eretmopodites silvestris* Ingram & De Meillon.
82. *Harpagomyia trichorostris* Theobald.
83. *Lutzia tigripes* Daruty & d'Emmerez).
84. *Megarhinus (Toxorhynchites) brevipalpis* Theobald.
85. *Mimomyia hispida* (Theobald).
86. *Mimomyia mimomyiaformis* (Newstead).
87. *Mucidus mucidus* Karsch.
88. *Mucidus scatophagoides* Theobald.
89. *Taeniorhynchus (Taeniorhynchus) aureus* Edwards.
90. *Taeniorhynchus (Taeniorhynchus) chubbi* Edwards.
91. *Taeniorhynchus (Taeniorhynchus) fuscopennatus* Theobald.
92. *Taeniorhynchus (Taeniorhynchus) metallicus* (Theobald).

93. *Taeniorhynchus (Mansonioides) africanus* Theobald.
94. *Taeniorhynchus (Mansonioides) mediolineatus* Theobald.
95. *Taeniorhynchus (Mansonioides) uniformis* Theobald.
96. *Theobaldia (Allotheobaldia) longiariolata* Macquart.
97. *Uranotaenia alba* Theobald.
98. *Uranotaenia bilaeniata fraseri* Edwards.
99. *Uranotaenia candidipes* Edwards.
100. *Uranotaenia fusca* Theobald.
101. *Uranotaenia mashonaensis* Theobald.
102. *Uranotaenia montana* Ingram & De Meillon.

3. MOSQUITO SPECIES FOUND AT ONDERSTEPOORT.

It was naturally impossible to conduct transmission experiments with every one of these 102 species and no useful purpose would have been served. Unlikely species had, therefore, to be eliminated. As already noted Onderstepoort could be regarded as a good place for horsesickness work and the natural transmitter of the disease could be expected to occur here in fair numbers at any rate. Species not present at Onderstepoort could therefore be disregarded.

During the course of the work, 24 species of mosquitoes were found towards the end of the winter of 1931 and summer of 1931-32.

In the mosquito traps, which will be described in the next chapter, the following 16 species were caught as adults:—

1. *Anopheles cinereus.*
2. *Anopheles mauritianus.*
3. *Anopheles squamosus.*
4. *Aëdes caballus.*
5. *Aëdes dentatus.*
6. *Aëdes hirsutus.*
7. *Aëdes lineatopennis.*
8. *Aëdes poweri.*
9. *Aëdes punctothoracis.*
10. *Culex annulioris.*
11. *Culex decens.*
12. *Culex fatigans.*
13. *Culex theileri.*
14. *Culex univittatus.*
15. *Lutzia tigripes.*
16. *Taeniorhynchus africanus.*

The following species were found in the larval stage in their breeding grounds:—

17. *Anopheles pretoriensis.*
18. *Aëdes argenteus.*
19. *Aëdes durbanensis.*
20. *Aëdes nigeriensis.*
21. *Aëdes vittatus.*
22. *Culex duttoni.*
23. *Culex salisburiensis.*
24. *Mucidus scatophagoides.*

Of these species, *Aëdes durbanensis* and *A. nigeriensis* were recorded from Onderstepoort and the Transvaal for the first time. The following were collected both as larvae and adults:—

Aëdes caballus.
Aëdes dentatus.
Aëdes hirsutus.
Aëdes linneatopennis.
Anopheles squamosus.
Culex theileri.
Lutzia tigripes.

It must be mentioned here that no special effort was made during this season to procure as many species as possible.

Besides the species mentioned above the following have previously been collected at Onderstepoort (cf Bedford 13th and 14th Rept. Dir. of Vet. Ed. and Res. 1928):—

25. *Anopheles argenteolobatus.*
26. *Anopheles ardensis.*
27. *Anopheles funestus.*
28. *Anopheles gambiae.*
29. *Anopheles maculipalpis.*
30. *Anopheles marshalli.*
31. *Anopheles natalensis.*
32. *Anopheles rhodesiensis.*
33. *Anopheles rufipes.*
34. *Anopheles theileri.*
35. *Anopheles demeilloni.*
36. *Aëdes cumminsi.*
37. *Aëdes quasiunivittatus.*
38. *Aëdes simpsoni.*
39. *Culex bitaeniorhynchus.*
40. *Culex pulchrithorax.*
41. *Culex quasigelidus.*
42. *Culex simpsoni.*
43. *Mucidus mucidus.*
44. *Taeniorhynchus fuscopennatus.*
45. *Taeniorhynchus uniformis.*
46. *Theobaldia longiareolata.*
47. *Uranotaenia candidipes.*

Altogether therefore, 47 species of mosquitoes belonging to the following 8 genera are known to occur at Onderstepoort up to the present:—

Anopheles	15	species.
Aëdes	13	,,
Culex	11	,,
Lutzia	1	,,
Mucidus	2	,,
Taeniorhynchus	3	,,
Theobaldia	1	,,
Uranotaenia	1	,,

The finding of 2 species of *Aëdes* during this survey not previously known to occur in the Transvaal indicates that this list might not yet be complete, although the more common species have certainly all been included.

4. RESULTS OF CATCHING MOSQUITOES BY MEANS OF TRAPS.

The number of mosquito species present at Onderstepoort is still far too large to make it practicable to use them all for transmission experiments. Further selection on epidemiological grounds is, therefore, still necessary.

In order to obtain some information as to the relative abundance of the mosquito species flying around at night, we used specially constructed traps, containing a horse as bait animal. Traps of this sort had already been used in the earlier mosquito work at Onderstepoort.

These traps consist of a wooden framework covered with thin mosquitoproof hessian and protected against rain by waterproof linen. This mosquito tent contains a horsebox into which a horse is put in the late afternoon, at 5 o'clock, the animal remaining there over-night. The entrance is closed in such a manner that a small vertical slit remains open between the two overlapping parts of the hessian forming the entrance. Mosquitoes, attracted by the animal can enter through this slit, but it is difficult for them to find their way out again. When looked for the next morning they are found resting under the roof or at the sides, often in some dark corner behind the wooden poles supporting the tent.

Two of these traps were placed some distance outside the laboratory. The first trap was erected towards the east near the Aapies river under some trees at the edge of an area of bushveld. The second trap was placed about two-thirds of a mile to the north of the laboratory on a small road sheltered by trees between the native location and a large portion of open bushveld surrounding the Bon Accord dam. In the neighbourhood, breeding places of *Aëdes caballus*, *A. lineatopennis* and *A. dentatus* were present.

On some occasions lights were kept burning all night in the traps with the intention of making them more attractive to mosquitoes. However, no constant differences between dark and illuminated traps were observed.

The first trap was ready for use on September 24th, and the second in the beginning of November, 1931. The use of both traps was discontinued on February 11th, 1932. A list of mosquitoes collected in these traps is given in Table 3.

Altogether 1,224 mosquitoes were caught, representing 16 species. Of these 21 specimens belonged to *Anopheles*, 683 to *Aëdes*, 517 to *Culex*, and 3 to *Taeniorhynchus* and *Lutzia* together.

The *Anophelines* were very rare, only 21 specimens belonging to 3 species, *A. cinereus*, *A. mauritianus* and *A. squamosus*, being found. The season, therefore, must have been very unfavourable for *Anopheles* breeding. Furthermore, *A. squamosus*, to which belonged two-thirds of the total number, is a river breeding species not depending directly on rainfall.

Of the 5 *Culex* species present amongst our material, *C. theileri* was the only common species. Nearly three-quarters of them were caught in October in the trap alongside the Aapies river, where they were breeding in large numbers. It is a species common throughout the year with no special summer preference.

TABLE 3.

List of Mosquitoes collected in Mosquito Traps at Onderstepoort between 24th September, 1931, and 11th February, 1932.

Month.	September.	October.	November.		December.		January.		February.		Total.
Trap.	I	I	I	II	I	II	I	II	I	II	I II
1. *An. cinereus*	—	3	—	—	—	—	1	—	—	—	4
2. *An. mauritianus*	—	—	—	—	2	—	2	—	—	—	4
3. *An. squamosus*	1	9	1	—	—	1	1	—	—	—	13
4. *Aëdes caballus*	—	—	1	13	—	5	—	1	1	—	21
5. *Aëdes dentatus*	—	—	77	107	17	65	10	11	62	96	455
6. *Aëdes hirsutus*	—	—	—	—	—	2	1	1	—	—	4
7. *Aëdes lineatopennis*	—	2	49	28	8	11	4	10	30	44	186
8. *Aëdes poweri*	—	—	—	—	—	4	1	1	—	—	6
9. *A. punctothoracis*	—	—	—	1	1	8	—	—	—	1	11
10. *C. annulioris*	—	—	—	—	3	—	—	1	—	1	5
11. *C. decens*	—	—	—	—	4	1	2	2	2	1	12
12. *C. fatigans*	—	—	—	—	3	4	1	—	1	—	9
13. *C. theileri*	9	327	13	15	48	29	25	8	—	14	488
14. *C. univittatus*	—	—	—	—	—	—	3	—	—	—	3
15. *L. tigripes*	—	1	—	—	—	—	—	—	—	—	1
16. *T. africanus*	—	—	—	—	—	—	2	—	—	—	2

Taeniorhynchus africanus was represented by 2 specimens, and *Lutzia tigripes* only by one. This latter species so far as we know, is not a bloodsucker.

The *Aëdes* species were the most numerous. In September 0 out of 10 belonged to them; in October, 2 out of 342; in November, 276 out of 305; in December, 121 out of 216; in January, 40 out of 77; and in February, 234 out of 253 specimens.

This distribution of the *Aëdes* species coincides very closely with the rainfall (cf. Table 1). The largest numbers were caught in November, this month, especially during its first 10 days, having had a good and well-distributed rainfall. December was very dry, only 1·65 inches of rain having fallen. The number of mosquitoes was reduced, but still remained fairly large, a fair number of those which hatched out in November undoubtedly still being alive. In January

the effect of the drought in December was well demonstrated by the extremely small number of specimens collected. On 20th and 21st January, more than 3 inches of rain fell and in February large numbers of mosquitoes re-appeared again. In 11 days' time nearly 6 times as many mosquitoes were caught as during the whole of January.

Of the 6 *Aëdes* species found, A. dentatus, with 455 specimens, was by far the most common. Then followed *A. linneatopennis* with 186 specimens, whereas of *A. caballus*, 21, of *A. punctothoracis*, 11, of *A. poweri*, 6, and *A. hirsutus*, 4 specimens were collected. This numerical distribution of specimens over the different species, however, could not coincide with the actual composition of the mosquito fauna in the field. On several occasions we were breeding out thousands and thousands of mosquitoes, especially *A. caballus* within a few hundred yards of our second trap, but notwithstanding this, only very few specimens could be recovered from the trap. *The traps therefore failed to give us an accurate indication of the relative abundance of the mosquitoes present in the field*, which was the main object of their use and that is the reason why further work with them was discontinued in February.

It is a well-known fact, that some species of mosquitoes regularly enter houses or stables at night, whereas others are only exceptionally found inside rooms. Our mosquito tent seemed to have the same effect on these insects as a house or stable. Some species were attracted by it and the bait animal inside and consequently were caught in large numbers. Other species only occasionally entered the traps and some others not at all. Horsesickness, however, is a disease transmitted in the field and only very exceptionally in stables. Thus, instead of giving us the information desired, *the traps merely showed a tendency to select those species, which were less likely to be connected with horsesickness.* This negative information, however, was also of some importance as we will see in the final section of this paper.

5. BREEDING PLACES AND BREEDING HABITS OF SOME MOSQUITO SPECIES FOUND AT ONDERSTEPOORT.

The most valuable information, so far as this could be expected in a not very favourable season, we hoped to obtain from a survey of the breeding places in the neighbourhood of the laboratory. To this we devoted quite an important part of our time.

The Onderstepoort Laboratories are built on what was formerly a farm about 8 miles distant from Pretoria near a small river, the Aapies river, which enters the Bon Accord dam. The Laboratory buildings, houses, stables, farm yard and some areas of cultivated land form a centre, which for a large part is surrounded by typical Transvaal bushveld, more or less untouched except by the grazing of animals. Just at the edge of this veld in particular we searched for breeding places and were fortunate in finding all the different conditions of the veld to be present within a few hours' walk, so that regular visits were possible without interfering too much with the experimental work.

In the accompanying map (Fig. 2) the site of the main breeding places in the neighbourhood of the Laboratory is shown.

A. Breeding Places of *Anopheles* Larvae.

Anopheline larvae were very scarce but were found from time to time together with those of *C. theileri* in backwaters of the river from October onwards. Most of them proved to be *Anopheles squamosus* and later in the summer a few larvae of *A. pretoriensis*

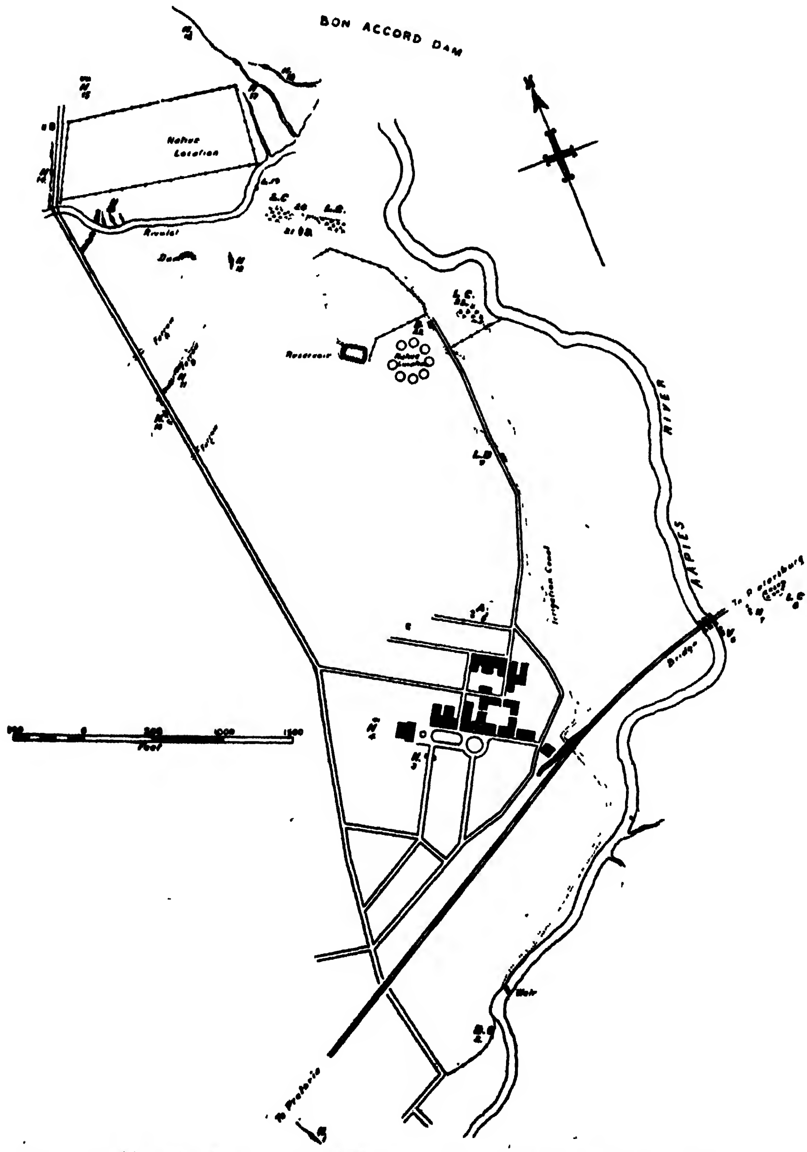

Fig. 2.—Map showing the *Aëdes* breeding places near Onderstepoort. The numbers are referred to in the text. A=*A. argenteus*, C=*A. caballus*, D=*A. dentatus*, H=*A. hirsutus*, L=*A. lineatopennis*, and V=*A. vittatus*.

were also collected. Small pools and animal hoof prints alongside the Aapies river and another small rivulet running at the foot of the new native location were investigated on different occasions but except for a few *Culex* larvae nothing was found.

It may be mentioned here that during the latter part of the summer *A. squamosus* was occasionally found breeding in a typical *hirsutus* pool (*vide* breeding place 11, next chapter).

B. Breeding Places of *Aëdes hirsutus*.

The first larvae of *Aëdes hirsutus* were found at the beginning of November, 1931, opposite the new native compound in breeding place 14 (cf. the map). A small furrow made by rain water runs down a slope alongside the road. After a rain, water remains for some time in a number of depressions between stones, and in these the larvae were caught. There were no trees or other vegetation except grass in the neighbourhood. After continued regular inspections this breeding place was not found to be too good.

Fig. 3.—Breeding place of *Aëdes hirsutus* in a furrow near a road. At Onderstepoort.

Quite a number of other breeding places were found in the vicinity of this first one, alongside the road between the laboratory and the new native location, and on each side of this location.

Breeding place 11 was situated alongside the road mentioned above in a depression immediately below a concrete culvert passing beneath the road (Fig. 3). It was surrounded by typical bushveld and could be regarded as a good breeding place. A water pipe running over this pool to which a tap was fitted enabled us to fill the pool with water at will.

On the 2nd of January, 1932, e.g. we filled the pool with tap water. On the 4th, 0·4 inch rain fell. On the 6th large numbers of small larvae were seen. The pool depended in the meantime practically solely on tap water. The first pupae appeared on 11th January in large numbers. They were frequent the next day, but thereafter their number decreased, on the 15th the last ones being collected. On that date the breeding place almost dried up but was immediately filled again. Small numbers of larvae reappeared on 21st January, but did not become numerous. On later occasions we were also able to produce larvae by filling the place with tap water and sometimes, but not regularly, obtained them in quite large numbers.

The eggs must have been present in the dried up pool for when larvae appeared at all, they did so after a few days and were nearly all of the same size. When water remained in the pool for some time *Culex* species deposited their eggrafts on the surface of the water, their larvae hatching out, but being quite small still at the time that the *Aëdes* were pupating. This fact also serves to indicate that the *hirsutus* eggs must have been present in some dormant state in the dried pool. Whenever we kept this under water for a longer period than sufficed for one generation of *hirsutus* to breed out no more larvae appeared, or at any rate only very small numbers. As it was not possible to keep the water level in the pool constant, these larvae might have originated from eggs lying in parts of the pool that had been dried up for some time. Occasionally some larvae of *Aëdes lineatopennis* and *Anopheles squamosus* were found breeding in the same pool besides the *Culex* already mentioned.

A few yards from this pool another breeding place of *A. hirsutus* (No. 10) was situated in the same furrow. It was very small and shallow, however, and only occasionally retained sufficient water to produce adults. On one occasion it was filled with clear water by a leakage from a cattle drinking trough and this also caused eggs to hatch out. In this pool the larvae were sometimes found associated with those of *Mucidus*.

Some really good breeding places were located between the native location and a small spruit or stream (No. 13). They conconsisted of a few medium-sized, elongated, shallow pools alongside a few large boulders. They were unsheltered although there were a few low shrubs in the vicinity, the surrounding veld consisting of sparse bushveld. After a good rain these pools became filled with muddy water and, provided they remained full for a sufficiently long period, regularly yielded a good supply of larvae.

Two other breeding places, situated close together, were found on the other side of the native location (No. 15, map). One consisted of a fairly deep old quarry, in which, however, larvae were only occasionally present. The other lay a few yards away in a very shallow depression covered for the most part by short grass. A tree standing in the vicinity afforded the only protection, otherwise the surroundings were quite bare. After a good rain the place would be filled with muddy water and fair numbers of *A. hirsutus* were frequently present. On 20th and 21st January, e.g. heavy rain fell, and on the 25th numerous small larvae were observed. Compared with others, this place, on account of its vegetation, was not a typical *hirsutus* breeding ground.

Typical breeding places were encountered again to the east of the native location in depressions formed in a number of small gulleys, conducting rainwater to the main stream, which traversed a slope covered with sparse bush (Nos. 16-18 of the map and another further south not shown). One of these pools in particular (Fig. 4) regularly produced larvae in fair numbers after every rain. Breeding places of this type, depressions in gulleys or furrows filled with water only after rains, were also found to the south-west of the laboratory (breeding place 1).

Another breeding place, which, during its short existence, supplied us with large numbers of larvae, lay near the main buildings of the laboratory (breeding place 3), alongside a road on either side of which depressions existed which occasionally became filled with rain water. Another similar place was situated behind the library (breeding place 4).

Fig. 4.—Breeding place of *Aëdes hirsutus*. A small pool in the veld filled with muddy water. At Onderstepoort.

A different type of breeding place was found near the Aapies river (breeding place 7), alongside an irrigation furrow which was generally full of water. This consisted of a number of small pools of the size of animal hoofprints and crab holes in which on one occasion a fair number of *hirsutus* were found.

At the beginning of March, 1932, we paid a short visit to the Government Irrigation Settlements at Losperfontein, about 40 miles west of Pretoria, where horsesickness had appeared amongst immunized mules at that time. Near Losperfontein *Aëdes hirsutus* was found breeding in a very remarkable manner. Water for irrigation and drinking purposes was conducted by a small furrow constructed

alongside a road. This furrow was provided with concrete intersections at regular intervals along its length. Under these intersections pools (Fig. 5) had formed by the washing out of the ground by the falling water, and in these pools, containing clear water, *A. hirsutus* was breeding in large numbers independent of the rainfall. This was a good example illustrating the necessity of taking precautions when water is led over open ground.

Fig. 5.—Breeding place of *Aëdes hirsutus*. Below concrete intersections in an irrigation furrow. Near Losperfontein.

Aëdes hirsutus *depends principally on rain for its larval hirsutus generally selects as breeding places*, at any rate in the Pretoria district, *small or medium-sized pools of muddy water without any vegetation, frequently lying in depressions in furrows made by rainwater. The pools are not sheltered but lie in more or less open veld.* The water in them remains muddy with a reddish or grey colour until it has practically dried up.

Pools with vegetation, mainly grass, are not disregarded entirely, although they certainly only constitute occasional breeding places.

Aedes hirsutus *depends principally on rain for its larval development. The eggs must be present in the pools before they are filled with rainwater. Furthermore, practically only one generation will hatch out in a breeding place if it remains flooded over a longer period than that necessary for the development of this one generation.*

When the breeding places are filled with clear water, larvae will hatch out in them, but clear water does not seem to be very attractive to the females as it was difficult to create new breeding places by filling suitable spots with tap water and allowing them to dry up from time to time.

The larval development is short, the first adults appearing about 8-10 days after the filling of the breeding places. The rate of larval development depends on the outside temperature and the size of the breeding places, small and shallow pools being warmer than larger and deeper places.

Adults were not observed in the neighbourhood of the breeding places except only directly after hatching from pupae.

C. Breeding Places of *Aëdes caballus* and *A. Lineatopennis*.

The first larvae of *A. caballus* were found quite unexpectedly in October, 1931. On that date larvae were observed in shallow water covering quite a large piece of open veld situated between the Aapies river and the old native location, just below some fields of maize (breeding place 23). The following day a small amount of water only was left which had completely dried up the day thereafter.

A week or so later larvae of *A. caballus* were found, again quite unexpectedly, in a furrow covered with grass situated near a plantation of trees at the edge of open veld, between the Aapies river and the new native location (breeding place 20-21). On returning the next day all the water had disappeared.

In both cases it was difficult to trace any connection between the appearance of the larvae and rainfall but on going into the matter we found that the ground had most probably been flooded by an overflow of water used for irrigating maize fields. On 4th November we once more saw larvae in the first breeding place and were then able to trace definitely the source of the water to the irrigation operations. Having established this fact, we connected both breeding places with the irrigation system of the farm lands belonging to the laboratory by means of small furrows (cf. map), in order to enable us to fill the breeding places at will and thus be independent of rain to some extent. One difficulty which confronted us, however, was to get sufficient water to the breeding spots during the spells of dry weather. The distance from the point where the water had to be pumped out of the Aapies river to our breeding places was nearly 1½ miles, and it took more than 24 hours for the water to reach its destination as very large quantities were absorbed by the dry ground of the furrows. On account of the high evaporation rate, water had to be led to the places over a period of several days, which would frequently interfere with the farming operations. As an example of the extreme dryness of the ground we may mention the following observation. On one occasion we endeavoured to fill one of the smaller breeding places (No. 15) with water by means of a water cart. 6,000 gallons of water were poured on to the same spot during one day but this was immediately all taken up by the ground. We were therefore forced to give up this idea.

Reverting back to the breeding places themselves, the best were the three marked in the map as 20-21 and already partially described (Fig. 6 and Fig. 7). The centre was formed by an old furrow covered with grass which, since the middle of January, had been connected with the main irrigation canals. The water coming down this

furrow was stopped by the remains of an old road slightly raised above the ground level. At this point the water flowed to either side of the furrow into open pans covered with grass but not sheltered in any way. Small dams and furrows were constructed so that we could dry up these pans alternately. A number of holes were dug so as to concentrate the water and larvae in them while the pans were drying up.

On 21st January the larger of the pans was filled by rainwater and during the following days irrigation water was added to prevent it from drying up. On the 26th very numerous larvae and pupae, about equal in number, were observed. Later on we found that 5-6 days was the ordinary period for the larval development during warm weather.

Fig. 6.—Breeding place of *Aëdes caballus* and *Aëdes lineatopennis*. An old furrow covered with grass. At Onderstepoort.

We were now able to expect enormous numbers of larvae with absolute certainty a few days after the place had been filled by rain or irrigation water. However, when the place was kept filled with water over a longer period than that necessary for the development of one generation of *Aëdes*, never would a second generation appear and only *Culex* would then continue breeding thereafter. Eggs of *Culex* were often observed on the water surface. By the time their larvae hatched out, the *Aëdes* were almost ready to pupate. The same holds good for *Lutzia tigripes*, whereas the larvae of *Mucidus* appeared together with those of *Aëdes*. There is no doubt that, as is the case with *hirsutus*, the eggs ready to hatch out are present in large numbers in the dried up pools.

The *Aëdes* larvae accumulated near the edge of the water especially between the grass stems. All the breeding places were covered with grass but in the deeper parts, where the grass was totally immersed, the larvae were less frequently found.

The duration of the larval period was nearly the same for the majority of the specimens, although variations of a few days occurred. The length of the pupal period seemed to be 1-2 days.

When the adults hatched out in these breeding places they appeared simultaneously in thousands so that, when walking alongside the edge of the water at that time, large swarms of mosquitoes, males and females together, were disturbed. They would fly for a short distance and then settle down again in the grass. Numerous visits showed us that these adults would remain at their breeding spots even after the water had disappeared, as the grass of these

Fig. 7.—Breeding place of *Aëdes caballus* and *Aëdes lineatopennis*. A pan in the veld covered with grass. At Onderstepoort.

breeding places retained moisture for a much longer time than the other parts of the veld. It is quite a simple matter to ascertain the presence of these mosquitoes by just walking through the grass, as large numbers are disturbed and will fly up, many settling down for a time on the body or clothes. Inside the comparatively small breeding area we were always able to find large numbers for at least a week, whereas outside this area, except when the whole veld was wet, very few if any were ever present. When the breeding ground itself commenced to dry out completely, the mosquitoes disappeared more or less slowly as the ground no longer offered them the humid conditions required. Some may have found other more suitable places, but the majority, during a prolonged period of drought, will undoubtedly have perished.

In these pans and in the furrow *Aëdes caballus* and *A. lineatopennis* always bred out together. *A. caballus* was the more prevalent species from the beginning, and towards the end of the season the percentage of *A. lineatopennis* still further diminished, so that eventually only very few specimens could be obtained, whereas the numbers of *A. caballus* remained practically unchanged. This may have been due to a seasonal adaptation of the species or to changes in the breeding places, produced by the repeated floodings with irrigation water.

Besides these two species and the other species previously mentioned *Aëdes nigeriensis* and *A. durbanensis* were forund in the same place towards the end of the season. The adults of *A. caballus* and *A. lineatopennis* would frequently attack us in broad daylight although they were definitely more active in the late afternoon. The mosquitoes did not really fly around during the day, but on being disturbed and settling down thereafter on the body, they immediately commenced feeding. The same observation was made with donkeys that frequently grazed over the breeding grounds.

The second place already mentioned (breeding place 23) also regularly yielded a large number of larvae, mainly *A. caballus* mixed with a smaller percentage of *A. lineatopennis*, when it was filled with irrigation water. It was, however, very shallow and dried up quickly and was therefore not as suitable for our purpose as the places described above. The adults behaved in the same manner. After hatching out they remained at their breeding place for a considerable time. Up to the middle of February a mosquito trap containing a horse as bait animal was used a few hundred yards away, but, as has been stated already, only very few *A. caballus* entered it. It would seem, therefore, that this species remains on or near its breeding grounds even at night.

A place, which produced *A. lineatopennis* exclusively, was found not far distant alongside the small stream running at the foot of the new native location and later joining the Aapies river (breeding place 19). Just at the edge of this stream a depression in the form of a small backwater had been formed. It was covered with fairly thick grass and after a rain would be filled with rainwater, but at the same time it became connected with the stream by the rising water. It was remarkable that no *caballus* were found there as conditions appeared to be favourable for this species.

In the water reservoir lying between the old native location and the open fields north of the first described breeding places, larvae of *Aëdes* were occasionally found. This reservoir was only filled with water for irrigation purposes from time to time. The first larvae were observed in it at the beginning of November, most of which belonged to *Culex* and a small number were *Aëdes caballus*, *lineatopennis* and *dentatus*. The dam did not prove to be a good breeding place for larvae of the latter genus however.

One of the breeding places first located by us lay between the road and an irrigation furrow (breeding place 9). A row of trees stood there and thick grass covered the whole area. After heavy rains the irrigation canal overflowed and pools were formed in the

grass under the trees. In November, when the place was first noticed, principally larvae of *A. lineatopennis* were found although besides them a few *A. hirsutus*, *caballus*, *dentatus*, *Mucidus*, *Lutzia tigripes* and large numbers of small *Culex* larvae were also discovered. The place was very small and dried up on most occasions before the larval development was accomplished, and apart from this, it did not produce larvae after every rain.

The last breeding place of this series (No. 8) was found at the beginning of January on the other side of the Aapies river. It consisted of a small clearing in fairly dense bushveld at the foot of a railway embankment and lay at a somewhat deeper level than the surrounding ground. After heavy rains it became filled with rainwater, augmented perhaps by an overflow from an irrigation furrow situated close by, and on several occasions contained fair numbers of larvae. These belonged mainly to *A. lineatopennis* and only a small percentage of *A. caballus*. The usual small *Culex* larvae were also present.

Conclusions.

From these observations the following conclusions may be drawn:—

Aëdes caballus and *A. lineatopennis* show a definite preference for certain types of breeding places. *They select furrows, small or medium-sized pans or slight depressions forming part of the veld, i.e. marshy spots covered with grass and filled with rainwater.* Contrary to *A. hirsutus* they prefer places with vegetation.

The larvae of both species develop equally well in rain or irrigation water. *On no account do they make use of permanent water for breeding and should the water in their most suitable breeding places become permanent, not more than one generation will appear therein. If, however, the place dries up for some time and becomes filled with water again, another generation immediately starts to develop.*

The eggs, ready to hatch, must be present in the dried breeding places. The contact with water probably provides the stimulus necessary for the hatching process. Not all the eggs seem to respond to this stimulus at the same time, however, as a number appears to remain unhatched when the place dries up again and perhaps the next contact with water will cause them to hatch. If this were not so, it would be difficult to explain the constant large numbers of larvae which hatch out every time the place is flooded, even when the intervening dry period was very short. This fact is of great biological importance to the species. If all the eggs were to hatch out after the first contact with water, practically all the larvae would be destroyed after some rains insufficient to permit of the adults emerging.

Both species must overwinter in the egg stage. In winter adults of the different *Aëdes* species were never encountered. When, however, at the beginning of October (before the adults appear), one of the breeding places was filled with irrigation water, large numbers of larvae appeared directly. The place dried up before the larvae

could complete their development, but on being flooded again, without any adults being present, larvae started to develop again. This can only be explained by the fact that the eggs were present in the places throughout the whole winter.

The larval development was very short, usually occupying 5-6 days in the summer months. With a pupal period of 1-2 days the adults therefore hatched out within a week after a good rain.

The adults, after hatching out, *remained at their breeding ground sheltering in the grass for a considerable period, even when the place had dried up in the meantime.* They were strictly confined to their breeding spots which retained a sufficient degree of humidity for a longer time than the rest of the veld. When, in cases of no further rain, the breeding grounds became absolutely dry, the mosquitoes disappeared from them as well.

Both species fed in broad daylight, if they were disturbed, and had settled down on the body. They did not, however, actually fly around hunting for food.

A. caballus and *A. lineatopennis* usually bred together. In some places one species was more prevalent than the other while in other places the reverse was the case. These differences remained constant throughout the season.

The larvae were more or less regularly associated with those of *Mucidus* and those of *Culex* and *Lutzia*, which, however, generally appeared later. On some occasions we encountered a few larvae of *Aëdes durbanensis, nigeriensis, hirsutus* and *dentatus* breeding with them.

D. Breeding Places of *Aëdes dentatus*.

The best breeding place for this species was found east of the railway line near the Aapies river (breeding place 2). It consisted of a small pond-like depression, a few feet in diameter under some trees, surrounded on all sides by thick bush. The place was very well sheltered. Apart from dead leaves the pool contained no vegetation, its bottom consisting of fine mud. After good rains the pool was filled with water and when the water remained long enough quite large numbers of *A. dentatus* bred out.

Another good place was found near the old native compound (breeding place 22). An old furrow of the irrigation system, which was no longer used, situated alongside an old road, was occasionally filled with waste water from the maize fields. In parts this furrow was well shaded by trees and bushes. The bottom consisted of fine mud mixed with fallen leaves. In these parts, together with the usual *Culex*, larvae of *A. dentatus* were found on several occasions in fair numbers.

Besides these places larvae of *A. dentatus* were found together with those of some other *Aëdes* species in a small shady place near one of the irrigation canals (breeding place 9), and in the water reservoir near the old native location. These places have already been described in the preceding chapter.

Conclusions.

According to our observations *Aëdes dentatus seems to prefer well sheltered and shaded pools without any vegetation.* We have never succeeded in finding its larvae in the open veld.

This species also depends on rain for its larval development, as permanent water was never found to be used.

We did not observe the adults near the breeding places. As stated before, they entered the mosquito traps in relatively large numersb at night, in fact they were the most abundant *Aëdes* species caught in them.

It seems, therefore, that A. dentatus *is a shadow or shelter liking species both in its larval and adult stages.*

E. BREEDING PLACES OF *Aëdes vittatus.*

Aëdes vittatus, a species belonging to the same subgenus as the yellow fever mosquito, was found in three places.

The first place (breeding place 6), was situated almost under a railway bridge crossing the Aapies river. On the top of a rock some 10 feet wide and 5 feet high, situated towards one side of the bed of the river, a depression, about 10 inches in depth, existed. After a fair rain this depression became filled with water which remained there for a number of days. From October we kept this place filled with river water for the greater part of the season, as it was known by one of us, from former observations, to be a suitable breeding place for *Aëdes vittatus*. At first *Culex theileri* only were present, but in December, larvae of *Aëdes vittatus* also appeared which were later sometimes associated with those of *Culex salisburiensis*.

The larvae of *A. vittatus* occurred more or less regularly in this pool. Periodical drying up and refilling of the place did not seem to be as necessary for them as for the other *Aëdes* species although hatching condition may possibly be more favourable when the place is regularly dried up.

The number of *A. vittatus* obtained from this place was not very great. From January onwards dragon-fly larvae, which preyed upon the mosquito larvae, were always present in this pool and it was practically impossible to get rid of them notwithstanding daily destructions, as the adult females continued laying eggs. The semi-permanent nature of the pool may possibly have attracted the dragon flies more than would have been the case of a pool under natural conditions.

Another breeding place of *A. vittatus* was found on the farm Kaalplaas near Onderstepoort. In this case the breeding place was of the same nature as the one described above, and consisted of a shallow depression containing rainwater on a large flat rock situated halfway up one of the hills. Quite a number of fullgrown larvae and pupae were present.

The third and last breeding place discovered was also formed by a rock pool. It was located, during a short mosquito survey carried out in March, 1932, on the Government Irrigation Settlements at Losperfontein. On one of the kopjes, which was fairly densely covered with thorn trees, a shallow depression filled with rainwater was observed on a medium-sized rock (Fig. 8). It contained a fair number of *vittatus* larvae and pupae. In the two latter cases no larvae of other species were present.

Fig. 8.—Breeding place of *Aëdes vittatus*. A rock pool on a kopje near Losperfontein.

Conclusions.

Aëdes vittatus appears to breed only in rock pools containing no vegetation. The larvae were never found associated with other *Aëdes* larvae, although occasionally *Culex salisburiensis* were present.

The larvae of *A. vittatus* must be capable of withstanding relatively high temperatures as, on a summer's day; these rock pools become extremely warm. A few preliminary experiments also showed us that the thermal deathpoint of these larvae is higher than that of other *Aëdes* and *Culex* species.

F. BREEDING PLACES OF *Aëdes durbanensis* AND *A. Nigeriensis*.

Larvae of these two species were only found in breeding places 20 and 21, where they were associated as has been previously mentioned, with larvae of *Aëdes caballus* and *A. lineatopennis*. They appear to have the same breeding habits as these two species.

The larvae began to appear towards the end of the season but never became common.

G. Breeding Places of *Aëdes argenteus*.

Larvae of this species were found in some concrete pig troughs near the stables of the laboratory (breeding place 5). They appeared there in April, towards the end of the season, but were not at all common.

H. Breeding Places of *Mucidus scatophagoides*.

The larvae of *Mucidus*, a remarkably large mosquito in both its larval and adult stages, are carnivorous, preying upon other mosquito larvae. They do not seem to attack other aquatic insects or at any rate mosquito larvae are their preferential food, and they have therefore to breed in association with other mosquito species.

The largest numbers of *Mucidus* larvae were found in the main *caballus* and *lineatopennis* breeding place (Nos. 20-21). There their numbers increased gradually and towards the end of the season they became quite common.

Apart from this, larvae of *Mucidus* were located in three other places (breeding place 1, 9 and 10), twice associated with *A. hirsutus* only (1 and 10), and in the other place (9), with *A. caballus, dentatus, hirsutus* and *lineatopennis*.

In all cases the larvae of *Mucidus* appeared together with those of the *Aëdes* species, whereas the larvae of *Lutzia*, which also lives on mosquito larvae, appeared later. The eggs of *Mucidus* must therefore have been present in the dried pools, and in its breeding habits this species is closely allied to the typical *Aëdes* species. It also does not breed in temporary water and should its breeding pool not dry up one generation only appears. In the main *caballus-lineatopennis* place, where most of the *Mucidus* larvae were found, conditions were extremely favourable on account of the continued experimental flooding, and it is therefore difficult to decide whether, if under natural conditions *caballus* places are more favourable for *Mucidus* development than *hirsutus* pools. That the *caballus* places are very suitable is certain and further, that *hirsutus* pools also accurately fulfil the larval requirements of this species is beyond doubt.

Adults of *Mucidus* were occasionally observed on their breeding grounds, but these had probably hatched out recently, as it would seem that they do not remain there as regularly or as long as the adults of *A. caballus* and *A. lineatopennis*.

Conclusions.

Mucidus scatophagoides *is a larvivorous mosquito resembling in its breding habits the typical* Aëdes *species.* It is often associated with the larvae of *A. caballus*, *A. lineatopennis* and with those of *A. hirsutus*, thus breeding in marshy spots of the veld covered with grass, and in pools of muddy water containing no vegetation.

As is the case with the typical *Aëdes*, this species breeds only in temporary water and the eggs are present in the dried places ready to hatch after the first rain.

I. Breeding Places of *Lutzia tigripes*.

Lutzia tigripes is another species predatory in its larval stage. It was found fairly often in a large variety of breeding places; in marshy spots with dense vegetation, in pools without vegetation, in concrete basins, in the open field and in sheltered places, in clear, muddy or quite dirty water. It makes use of stagnant water, permanent as well as temporary, and was found together with *Aëdes caballus*, *A. lineatopennis*, *A. dentatus*, *Culex theileri*, *C. duttoni* and *Mucidus*.

In temporary pools the larvae of *Lutzia* always appear a few days later than those of *Aëdes* and therefore together with the *Culex* larvae. The eggs are therefore not present in the dried pools but are deposited after the places have been filled with water. In its breeding habits *Lutzia* resembles the true *Culex*.

Lutzia tigripes does not appear to be a bloodsucking species according to the literature, and in our experiments it could also never be induced to feed on horses. In connection with the transmission of horsesickness this species was therefore of no practical importance.

H. Breeding Places of *Culex theileri*.

Culex theileri is probably the most common mosquito species at Onderstepoort throughout winter and summer.

When we commenced our search for breeding places, at the beginning of September, 1931, the bed of the Aapies River was the only place where larvae of mosquitoes could be expected. Larvae of *Culex theileri* were found in fair numbers at the edge of the water, mainly in backwaters or in pools alongside the river. They were present quite regularly, excepting after heavy rains when they were washed away by the rising water. In the river the larvae seemed to be more abundant towards the end of winter than in summer.

Later on in the summer larvae were also found in a large variety of pools of a permanent or temporary nature. Larvae frequently appeared in typical *Aëdes* places, always later, however, than the latter species.

I. Breeding Places of *Culex duttoni*.

Larvae of *Culex duttoni* were observed in only one place near the Aapies River. Waste water from the laboratory, which usually ran into the river, was diverted and used for flooding a small piece of ground covered with grass on which a few adults of *A. lineatopennis* had occasionally been found. This latter species, however, did not develop there. Besides *Culex theileri* and large numbers of *Lutzia tigripes* larvae of *Culex duttoni* appeared and were quite plentiful from January onwards. The water filling this place was very dirty.

6. RESULTS OF THE MOSQUITO SURVEY IN CONNECTION WITH THE TRANSMISSION OF HORSESICKNESS.

Before going into a discussion of the results of our mosquito survey it would seem advisable to try and obtain some information from other diseases related to horsesickness itself or showing similarities in the seasonal distribution. There are four diseases which may be of use for comparative purposes, viz., bluetongue of sheep, yellow fever, dengue and malaria.

Bluetongue certainly shows affinities to horsesickness. Both are non-contagious virus diseases and have a similar distribution in South Africa. The transmission of this disease is, however, not yet known.

Yellow fever and *dengue* are virus diseases of man. The viruses, especially in the case of yellow fever, are very similar to that of horsesickness in quite a number of respects. Points of dissimilarity also exist, but of all the diseases we know, yellow fever seems to be the most closely related to horsesickness. Yellow fever and dengue are transmitted mainly by *Aedes argenteus*. Besides this species a number of other mosquitoes, chiefly belonging to the *Aëdes* group, has been found to be capable of transmitting yellow fever experimentally in recent years, whereas dengue is transmitted by at least one other *Aëdes* species.

The seasonal appearance of *malaria* in the Union shows a definite correlation with that of horsesickness. Bad malaria years are bad horsesickness years as well, both diseases being limited to the summer months and depending in their severity on the amount of rainfall. It must be mentioned, however, that geographically the two diseases differ, malaria being more restricted.

Horsesickness cannot, however, be transmitted by the same species of mosquitoes that carry malaria and yellow fever. The two malaria transmitting *Anopheles* species of the Union, *A. gambiae* and *A. funestus*, are house-frequenting species, whereas horsesickness is not generally contracted in stables but in the field. Apart from this, the geographical distribution of the two diseases, as previously mentioned, is not entirely the same. *Aëdes argenteus*, the yellow fever mosquito, can also not play an important part in the transmission of horsesickness. It lives in close association with houses and being mainly a tropical and sub-tropical species its distribution in the Union is more restricted than that of the horsesickness areas.

In analogy with malaria and yellow fever, we can therefore expect a species of Anopheles *or* Aëdes, *but a true field variety of these species, to be the transmitter of horsesickness and bluetongue.*

In trying to obtain information as to the probable transmitter of horsesickness from the results of our *mosquito survey*, we must not lose sight of the fact that *the season under review was not a typical horsesickness season at all.*

The *Anophelines* were very scarce this season. Only a few adults were obtained in the mosquito traps and only a small number of larvae could be collected. In fact, out of the 13 species of *Anopheles* known to occur at Onderstepoort, only 3 were found this season. *A. squamosus*, the only species of which a somewhat larger number and of which both larvae and adults were collected, is not limited to the summer months. It is, therefore, not likely to play any rôle in the transmission of horsesickness.

We were not able, by the experimental flooding of places regarded as possible breeding spots or by any other means, to create conditions favourable for the breeding of *Anopheles*.

The lack of information in our mosquito survey regarding the Anophelines *certainly presents a flaw*, but we had very little chance of getting more complete information.

Of the Culicine tribe, species of *Aëdes*, *Mucidus*, *Culex*, *Lutzia*, *Taeniorhynchus*, *Theobaldia* and *Uranotaenia* are present at Onderstepoort.

L. tigripes, representing the genus *Lutzia*, does not appear to be a bloodsucker and is, therefore, of no further interest in connection with this work.

Regarding the biology of the *Taeniorhynchus* species, *Theobaldia longiareolata* and *Uranotaenia candidipes*, we were able to obtain practically no data from Onderstepoort. During this season only a few adults of *Taeniorhynchus africanus* were caught in the mosquito traps. It seems rather unlikely that one of these 4 species would be connected with the transmission of horsesickness.

The genus *Culex* is represented at Onderstepoort by 11 species of which 7 were collected as larvae or adults during this survey. Of only 3 of the species, viz., *C. theileri*, *C. duttoni* and *C. salisburiensis*, were the breeding places located. *Culex theileri* was very common in the adult and larval stages, but as it was also present in large numbers in the winter, it can therefore not possibly belong to the list of the possible transmitters. The other species appeared in December, but as regards their distribution, not sufficient information was obtained. The larvae of *C. duttoni* became more common towards the end of the summer but they were only found in an artificial breeding place, which had not existed throughout the greater portion of the season.

According to our observations the *Culex* species made free use of permanent water for breeding purposes, possibly even in preference to temporary water. As the eggs of *Culex* cannot withstand complete dessication, and, on account of the embryonic development of the larvae always occupying a number of days, many temporary water pools are not suitable for their breeding as these places dry up before the larval development can be completed.

Our information was certainly far from complete, but nothing pointed to a *Culex* species as being probably involved in the transmission of horsesickness.

Of the 13 *Aëdes* species, known to occur at Onderstepoort, 10 were found during this survey, viz., *A. argenteus*, *A. caballus*, *A. dentatus*, *A. durbanensis*, *A. hirsutus*, *A. lineatopennis*, *A. nigeriensis*, *A. poweri*, *A. punctothoracis* and *A. vittatus*. In the case of 8 of these species the breeding places were located. We had thus gained quite valuable information so far as the *Aëdes* species were concerned.

A number of these Aëdes *species fitted in very well with the epidemiology of horsesickness.* To start with, all the *Aëdes* species studied, *depended entirely upon rainfall.* If there is no rain no mosquitoes will develop, and their numbers increase in accordance with the amount of rain. They do not make use of permanent water for breeding purposes, not even during a very dry part of the season when no temporary water is available. The eggs are present in the dried up pools ready to hatch when these pools become filled with rain-water. Larval development is accomplished within a week during the warm summer months. Should a breeding place dry up for a short time and become flooded again, another generation immediately commences to appear, but it is never followed by a second generation without intermediate drying up.

Of the 8 *Aëdes* species found this year, *A. argenteus* may not be regarded as a transmitter on account of its restricted distribution and special habits, as has been mentioned already.

Aëdes hirsutus has to be reckoned amongst the potential carriers. It was quite common throughout the season and bred mainly in small to medium-sized pools of muddy water containing no vegetation. After a good rain plenty of these pools are formed along the sides of the roads and in the veld. In practically all the temporary water courses formed after good rains pools of this nature remain. filled with sufficient water for breeding purposes. Water may also be added artificially in certain circumstances and in this way adults may be obtained independent of the rain (cf. Losperfontein). This is, however, rather the exception than the rule.

On account of the nature of its breeding places *A. hirsutus* is bound to be one of the most common of the *Aëdes* species. A very wet season is not necessary for its development as even a moderate rain is generally sufficient. This fact may therefore, to some extent, militate against the importance of its rôle as a probable transmitter.

Aëdes caballus and *A. lineatopennis* may be considered together. Although belonging systematically to two different sub-genera, they are biologically closely related to one another. They breed in marshy spots in the veld, by preference in small or medium-sized pans, in small depressions or temporary furrows, provided these are covered with grass.

We were able to breed these two species, especially *A. caballus*, in very large numbers, but only under semi-artificial conditions by flooding their ordinary breeding grounds regularly with irrigation water. Normally these pans are filled with water only after heavy rains, and more rain is required later in order to enable them to retain sufficient water until the larval development is accomplished. We feel convinced that without these artificial floodings *A. caballus*

especially, would have been rare. *A. linneatopennis*, which is able to make use of small breeding places as well, is therefore able to breed with less rain, but heavy rains extend its breeding places considerably.

In order that *Aëdes caballus* and *A. lineatopennis* may become really common large amounts of rain are required. A really wet summer is therefore necessary, so that in this respect these two species conform with the requirements of the probable transmitter.

Both species are not exclusively dependent upon rain, however. They may hatch out equally well, as we have seen, in the waste water from irrigation systems. Places filled in this way seldom remain filled long enough, however, to make the completion of larval development possible. In this way, outbreaks during dry seasons may possibly be explained. Further, in the behaviour of the adults of these two species, certain points exist which tend to incriminate them in the list of possible transmitting agents. We have seen that these species, especially *A. caballus*, remain, to a very great extent, in the immediate vicinity of their breeding places day and night. On farms where horsesickness occurs, often only certain parts of such farms, mainly vleis, where the important breeding places are to be expected, are regarded as dangerous, whereas on other parts of these same farms horses may be allowed to graze almost with impunity.

On account of their breeding habits, their dependence on large amounts of rain, and the behavious of the adults, Aëdes caballus *and* A. lineatopennis *must be seriously regarded as potential transmitters of horsesickness.*

Aëdes durbanensis and *A. nigeriensis* have the same breeding habits as the two former species but are less common. They will also therefore belong to the list of potential transmitters.

Aëdes vittatus has also to be reckoned amongst the possible transmitters. It only breeds in rock pools, of which quite a number must exist in most of the horsesickness districts. Such pools may be present in the vleis as well but are, however, more common on or near the kopjes. This fact, to some extent, goes against their rôle as possible transmitters, but they can certainly not be excluded from this list.

Aëdes dentatus is one of the common species of this genus. It breeds in sheltered pools without any vegetation. On account of its breeding habits it may be regarded as nearly as good a possible transmitter as *A. hirsutus*. It entered our mosquito traps relatively frequently in this way showing a preference for stable-like buildings. This fact is not in favour of its being an important transmitting agent, and for this reason this species must be regarded as only of secondary importance.

Aëdes cumminsi, A. poweri, A. punctothoracis, A. quasiunivittatus and *A. simpsoni*, the remaining species known to occur at Onderstepoort, seem to be more or less rare species and will probably not rank amongst the important potential transmitters.

Lastly, *Mucidus scatophagoides* is regarded in the literature as being a non-bloodsucking mosquito. Only towards the end of the season did we discover that this statement was not correct, as adults fed readily on horses and took up a surprisingly large quantity of blood.

Mucidus uses the breeding places of *Aëdes caballus*. *A. lineatopennis* and *A. hirsutus* and has to rank with these species amongst the potential transmitters. As it feeds on the larvae of these species it is usually less common. Large numbers of *Mucidus* can only be expected from the larger *caballus* pools after heavy rains, and it appears to become frequent only towards the end of the season, after a sufficient number of generations of the species on which it preys have developed. It must, therefore, certainly be regarded as one of the important potential transmitters.

The transmitters of horsesickness must of necessity have a fairly wide *geographical distribution* as the disease itself occurs over the larger part of Africa.

We will give here the geographical distribution of the species of *Aëdes* known to occur at Onderstepoort, so far as it is known in the countries where horsesickness is endemic.

A. argenteus.—Transvaal, Natal and Zululand, Rhodesia, Tanganyika Territory, Uganda, Nyassaland, Belgian Congo, Nigeria, Sudan and Egypt.

A. caballus.—Transvaal, Natal and Zululand, and Kenya.

A. cumminsi.—Transvaal, Natal and Zululand, Kenya, Uganda, Nyassaland, Belgian Congo (var.).

A dentatus.—Transvaal, Natal and Zululand, Kenya, Uganda, Belgian Congo and Sudan.

A. durbanensis.—Transvaal, Natal and Zululand, Kenya and Uganda.

A. hirsutus.—Transvaal, Natal and Zululand, Rhodesia, Kenya and Uganda.

A. lineatopennis.—Transvaal, Natal and Zululand, Rhodesia, Kenya, Uganda, Nyassaland, Belgian Congo, Nigeria and Sudan.

A. nigeriensis.—Transvaal, Kenya, Uganda, Nyassaland and Nigeria.

A. poweri—Transvaal, Natal and Zululand, Rhodesia, Kenya, Belgian Congo.

A. punctothoracis.—Transvaal, Natal and Zululand, Rhodesia and Belgian Congo.

A. quasiunivittatus.—Transvaal, Natal and Zululand, Rhodesia, Tanganyika Territory (? var.), Kenya, Uganda, Nyassaland and Nigeria.

A. simpsoni.—Transvaal, Natal and Zululand, Tanganyika Territory, Kenya, Uganda, Nyassaland, Belgian Congo and Sudan.

A. vittatus.—Transvaal, Natal and Zululand, Rhodesia, Uganda, Belgian Congo, Nigeria and Sudan.

Besides these species the following have a wider distribution over Africa: *Aëdes africanus* was found in Rhoedsia, Uganda, Belgian Congo and Nigeria *A. apicoargenteus* in Uganda, Belgian Congo and Nigeria. *A. argenteopunctatus* in Rhodesia, Uganda, Nyassaland, Belgian Congo, Nigeria and Sudan. *A. metallicus* in Natal and Zululand, Tanganyika Territory and Sudan. *A. ochraceus* in Rhodesia, Kenya, Nigeria and Sudan. *A. tarsalis* in Natal and Zululand, Uganda and the Belgian Congo.

From this list it is obvious that *those species regarded* as potential transmitters have a wide distribution over Africa.

SUMMARY.

Our present knowledge of the epidemiology of horsesickness strongly suggests that mosquitoes are the probable transmitters of the disease.

In order to obtain preliminary information as to the mosquito species most probably involved in its transmission and that of bluetongue in sheep, a mosquito survey was carried out at Onderstepoort during the summer 1931-1932.

The summer in question did not represent a good horsesickness season on account of the extremely dry weather conditions which prevailed. The total amount of rain from November to April was 18·40 inches, compared with 36·91 and 31·47 inches in good horsesickness years (cf. Graph 1).

In South Africa up to the present 102 species of mosquitoes, belonging to 13 different genera, have been found. At Onderstepoort 47 species are known to occur. During this survey 24 of these species were found, 16 as adults, 7 both as larvae and adults, and 8 only in the larval stage.

Using tent-like traps with horses as bait animals, between the end of November, 1931, and the middle of February, 1932, 1,224 mosquitoes were caught, representing 16 species. Of these 21 specimens eblonged to *Anopheles*, 683 to *Aëdes*, 51 7to *Culex* and 3 to *Taeniorhynchus* and *Lutzia*.

The *Anophelines* were exceptionally rare. Amongst the *Culex*, *C. theileri* was the only common species, most of the specimens of this species being caught in October.

The *Aëdes* appeared in October and were common, especially in November and February. There existed a definite relation between their numbers and the amount of rain.

The traps, however, failed to give correct data as to the relative abundance of the *Aëdes* species present in the field, as they were selective for certain species (*A. dentatus*) and unattractive for others (*A. caballus*). The use of the traps was consequently discontinued later.

A considerable time was spent in a survey of the *breeding places* of mosquitoes near Onderstepoort.

Larvae of *Anopheles squamosus* and *A. pretoriensis* were found in backwaters of the Aapies River, the former species sometimes being encountered in one of the *Aëdes hirsutus* pools as well. Anopheline larvae were exceptionally scarce throughout the whole season.

All the *Aëdes* species studied depend mainly on rainfall for their larval development. They make use only of temporary water for breeding purposes and even in a suitable place one generation only will develop so long as the place remains flooded. When such a place has dried up and becomes filled again, another generation appears. Permanent water is never used by them for breeding even should there be no temporary water available for a long time.

The eggs are present in the dried-up pools and the larvae hatch out within a very short time after such pools become filled with water. It appears that on contact with water only a certain percentage of the eggs hatch out, the remainder lying dormant until the place has dried up and is filled again.

The *Aëdes* species pass the winter in the egg stage. In the early spring and summer, without adults being present, large numbers of larvae were observed developing after their breeding places had become filled with water.

Larvae of *Aëdes hirsutus* were quite common after good rains and were found mainly in small to medium-sized pools of muddy water containing no vegetation. Pools of this nature are present in large numbers after good rains.

The larvae of *Aëdes caballus* and *A. lineatopennis* live for the most part associated with one another. They select marshy spots in the veld covered with grass, small to medium-sized pans, furrows or slight depressions in the veld. Most of these places are filled with sufficient water only after heavy rains. Larval development will also take place, however, when the breeding places are filled with irrigation water. By this means we were able to breed these species in large numbers independent of the weather conditions.

The adults of both species remain for a considerable period, even during the day, at their breeding grounds, sheltered in the grass. They are strictly confined to these places which usually retain a sufficient degree of humidity for a longer time than the rest of the veld.

Both species, when disturbed, bite in broad daylight, but do not actually fly around during the day hunting for food.

A. caballus in particular, was not attracted to any extent during the night by horses kept in tent-like stables erected in the neighbourhood of one of its breeding places.

Aëdes dentatus prefers well sheltered pools under trees and bushes with decomposed vegetation for breeding. In the open field its larvae were never found.

Aëdes vittatus breeds only in rock pools which do not contain any vegetation. The larvae are capable of withstanding relatively high temperatures.

Aëdes durbanensis and A. nigeriensis were found associated with *A. caballus* and *A. lineatopennis*. They are comparatively rare species at Onderstepoort.

Larvae of *Aëdes argenteus* were found in concrete pig troughs near stables. They appeared only towards the end of the season and were not at all common.

Mucidus scatophagoides, whose larval prey upon other mosquito larvae, resembles in its breeding habits the typical *Aëdes* species.

It was found associated with the larvae of *A. caballus*, *A. lineatopennis* and *A. hirsutus*, thus breeding in marshy spots covered with grass in veld, and in pools of muddy water containing no vegetation. It only uses temporary water for breeding and therefore also depends upon rainfall.

Culex theileri was common during both winter and summer. Larvae were found in backwaters of the Aapies River and also in a large number of places with permanent and temporary water.

Larvae of *Culex duttoni* were observed in one more or less permanent pool covered with grass.

Larvae of *Lutzia tigripes*, another species predatory in its larval stage, were found quite often in a large variety of breeding places with temporary and permanent water. It resembles *Culex theileri* in its breeding habits.

From the results of this survey we are able to draw the following conclusions:—

Anophelines may be connected with the transmission of horsesickness, their scarcity, during the season under review, indicating their dependence upon rain. Information as to the species probably involved could not be obtained.

The seasonal (not geographical) distribution of malaria in South Africa suggests that the transmitters of both diseases depend on the same climatic conditions. The malaria carrying species themselves cannot, however, be transmitters on account of their house frequenting habit.

A number of *Aëdes* species fit in very well with the epidemiology of horsesickness. Most of the species depend upon rainfall and breed only in temporary pools and their numerical appearance is thus proportionate with the amount of rain. Should any of these species be involved in the transmission the most probable transmitters would be *A. caballus*, *A. lineatopennis* and *A. hirsutus*. The two former species, the adults of which remain throughout their lives near the breeding grounds in the vleis, coincide most accurately with the epidemiology of horsesickness and bluetongue.

Aedes vittatus, *A. nigeriensis*, *A. durbaneneis* and *A. dentatus* must be regarded as potential transmitters of secondary importance.

These species all show a fairly wide geographical distribution over Africa.

The disease which seems to be most closely related to horsesickness is yellow fever in man, and it is transmitted chiefly by *Aëdes* argenteus, which fact is a further indication that *Aëdes* are good potential carries of the disease under review.

Mucidus is biologically very closely related to the *Aëdes* species and must also be regarded as an equally good potential carrier as *A. caballus* and *A. lineatopennis.* Contrary to statements in the literature this species is a voracious bloodsucker.

It is unlikely that *Culex* species would be involved in the transmission of horsesickness and bluetongue as they make frequent use of permanent water for breeding purposes. Furthermore, the most important species of this genus are not restricted to the summer months.

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Handling Mosquitoes for Experimental Purposes under South African Conditions.

By

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WHILE engaged in work on the transmission of horsesickness and bluetongue we commenced our experiments with mosquitoes and more especially with different *Aëdes* species, which, for reasons stated in the previous paper had to be regarded as the most probable transmitters. First of all suitable methods had to be evolved for rearing these insects, feeding them on the experimental animals and keeping them alive for a sufficient period under South African conditions. That there are typical South African conditions is shown at a glance from the following experiences:

When putting mosquitos contained in a small wire cage covered with mosquito netting, on stabled horses in Europe for one night, on an average, perhaps 70 per cent. will have fed and only a few, if any, will have died by the next morning. Repeating the same experiment in South Africa during a dry summer night, one will generally find, that the next morning 80-90 per cent. of the mosquitoes are dead and only a few specimens engorged. The adverse climatic conditions are responsible for this and this has proved to be the most important obstacle in any mosquito work out here.

We had to work out our methods from practically the beginning, as there were no former experiences on these lines of any real value. In fact, every earlier attempt to work with mosquitoes in connection with horsesickness had to be given up on account of technical difficulties.

We started our experiments with practically no equipment at all and everything had to be specially designed and constructed. The fact that the methods we used had to be altered from time to time during the course of the work to fulfil the requirements of the changing climatic conditions was unavoidable.

We feel sure that the methods finally adopted are not absolutely ideal and further improvements can certainly still be made, but they enabled us, at any rate, to carry out the main transmission experiments. This work being limited, as it was, to one season only, had to be tentative in nature as it was impossible to predict the influence of the climatic conditions of the whole season upon the mosquitoes under experiments conditions, on account of our being able to devote only

part of our time to these problems. We were, therefore, not able to investigate thoroughly the reasons for every failure or unsatisfactory result. Apart from this, on account of the expense involved, we had to give some of the elaborate apparatus, which took months to make, a thorough trial before suggesting further alterations.

A somewhat complete description of our technical observations and results seems to be indicated as a guide for further work on these problems.

We wish here to express our thanks to Mr. Appleton, who most ably assisted us in constructing the cages, stables and other devices used during this work.

1. CATCHING OF ADULT AND LARVAL MOSQUITOES.

The mosquitoes used in our experiments were collected as larvae and pupae or as adults.

The *larvae* and *pupae* were caught in the usual manner by means of a strong piece of wire bent at one end in the form of a loop about 4-5 inches long by 2-3 inches broad, which was covered with a double layer of ordinary mosquito netting. As a rule, only nearly fullgrown or fullgrown larvae and pupae were collected, as these required little attention in the laboratory.

The larvae and pupae were kept, until the hatching of the adults took place, in large glass jars, by preference, in water from their breeding places, as this supplied them with the necessary food. Extra food was usually not necessary, but in cases where a supply of food seemed to be wanted, powdered rice was found to be suitable as it was readily taken up by most of the larvae and only seldom caused putrefaction of the water. Overstocking of the jars must be avoided, as this, apart from giving rise to a shortage of food, leads to retardation of development or a high mortality, due to the amount of excreta passed into the water.

Care must be taken that all carnivorous insect larvae are eliminated from the jars. Beetle larvae may cause serious losses, but the most important, in our experience, were brought about by the larvae of two mosquitoes, *Mucidus scatophagoides* and *Lutzia tigripes*, which are often associated with the larvae of *Aëdes caballus*, *A. lineatopennis* and *A. hirsutus* in their breeding places, and which may devour a surprisingly large number of these larvae. In one experiment in the laboratory one larva of *L. tigripes* devoured, during the 10 days of its development, 43 larvae of *A. hirsutus*, viz., 16 halfgrown larvae during the first 4 days and 27 fullgrown during the last 6 days. The larvae of *Mucidus*, which are much larger than those of *Lutzia*, will require even more larvae.

The jars containing the larvae were covered with mosquito netting to prevent the newly hatched adults from escaping. For catching the adults the jars were placed in front of a window, the light attracting the insects to one side, part of the netting was lifted up and the mosquitoes caught in small tubes.

The mosquitoes had to be collected within a day of their hatching out as, especially when large numbers were hatching out together, they were liable to be drowned. When many mosquitoes had to be handled at the same time, a number of jars containing the larvae were sometimes placed together without cover in a sufficiently large cage. The number of drowned specimens was thereby considerably reduced and the catching of the mosquitoes could be extended over a number of days.

For catching and handling the mosquitoes, small test tubes, 6 inches long by ⅝ inch wide were found to be the most suitable. They are just large enough to allow the mosquito to enter, and small enough to prevent the insects from escaping easily. Large test tubes are not suitable on account of the ease with which the insects escape.

For catching *adults in the field* large hand nets were used with which the grass of the breeding grounds was swept. Catching the mosquitoes by means of tubes between the blades of grass was very difficult. Of an afternoon, when no strong wind was blowing, we found that the best method was often to wait until the mosquitoes, disturbed by our walking through the breeding grounds, settled down on ourselves or the boys. They were then easy to catch by means of tubes. This had the further advantage that only undamaged specimens, ready to feed, were caught. In this manner we obtained the adults of *Aëdes caballus* and *A. lineatopennis* in large numbers and besides them some *A. durbanensis* and *A. nigeriensis*. The resting places of the other mosquitoes were not located. It may be mentioned that walking through the country and sweeping the grass here and there, is very seldom of any use as, at least in the case of *A. caballus* and *A. lineatopennis*, the resting and shelter places are very restricted to certain spots, as has been stated in the review of the results of our mosquito survey. When walking through a typical resting place, the mosquitoes are disturbed and fly up in large numbers, when they are easily noticed.

The greatest difficulty encountered was the collection of sufficient material this season due to the shortage of rain and consequent scarcity of natural breeding places. Anophelines were very rare throughout the season and no way of assisting their breeding by artificial means could be found. The number of *Aëdes* occurring naturally was also not very large. However, we were able to make provision for some species at any rate, especially *A. caballus*, *A. lineatopennis* and *A. hirsutus*, by flooding known breeding grounds either with tap water or with irrigation water. The results were, in most cases, very satisfactory indeed, but owing to the general shortage of water we were unable to resort to this method as often as we wished. The methods employed and the difficulties in getting the water to the right spots have already been discussed in the preceding paper.

2. METHODS FOR KEEPING MOSQUITOES ALIVE IN THE LABORATORY.

The mosquitoes, which had hatched out from larvae, been collected in the field or had been caught as adults, had to be kept in the laboratory for some days to start with before they could be fed on an infected animal, and thereafter, until they were again put on to susceptible animals.

Freshly emerged adults do not feed directly. Their chitin must first harden thoroughly. From the second to third day after their hatching out they can be used for experiments. They had often, however, to be kept longer before use as only after certain intervals could animals be infected to feed them on.

Horsesickness is most likely not transmitted mechanically by mosquitoes, supposing these insects are the natural carriers. In all probability some sort of development, or at least multiplication of the virus has to take place in the mosquitoes before they become infective. In analogy with other diseases, yellow fever of man for instance, this incubation period in the insects is stated to last 10-15 days. It was therefore necessary to keep the mosquitoes alive for at least this period, preferably longer.

As is the case with most insects, *temperature*, *humidity* and *food* are the essential factors on which the mosquitoes depend. We knew that former experiments at Onderstepoort with mosquitoes had failed on account of their dying rapidly in captivity, and we had therefore to go somewhat deeper into these problems.

The mosquitoes require a certain *minimum temperature* for their existence and ordinary room temperature would have been sufficient during the summer months. However, the development of the virus would very probably be influenced by the temperature to a considerable extent. A certain (yet unknown) minimum will be necessary for the development to take place at all, whereas with increases in temperature the incubation period in the mosquitoes will be proportionately shortened. A high temperature was the most suitable for the virus, whereas at relatively low temperatures the mortality amongst the mosquitoes was the smallest. The optimum had to be regarded as the highest temperature just not detrimental to the mosquitoes. This optimum seems to lie in the neighbourhood of 26-28° C. We had at our disposal a warm room, not specially built for entomological purposes however. It was electrically heated but the heating had to be regulated by hand so that a constant temperature was therefore not obtainable. During the winter and early summer months the heating was on day and night, giving an average temperature of 24-26° C. During the later summer months the room was only heated during daytime as no control could be exercised at night and sometimes very high temperatures were recorded. As far as possible the temperature was maintained at an average of 26° as, on account of the insufficient means of control, an average of 28° was regarded as too risky. From time to time the temperature varied between 20 and 30° C.

To provide the mosquitoes with the necessary degree of humidity they were stored, in the first series of experiments, in ordinary jam jars, the lids of which were replaced by mosquito netting. Ten specimens were put into each jar, which was also provided with some strips of filter paper. The jars were kept on wet cotton wool in slightly larger jars provided with metal lids. These lids had holes in them to allow of the escape of excess water vapour, as absolutely saturated air is unfavourable for the mosquitoes. The mosquitoes were fed on sugar water, a small piece of cotton wool soaked in a 10 per

cent. solution being placed on the netting covering the jars. The jars had to be changed from time to time when they became soiled by the excretions of the mosquitoes or when deaths occurred. This method of keeping mosquitoes worked quite satisfactorily, especially when the mosquitoes were needed for injections and when only a small number had to be handled. Difficulties, however, were encountered with large numbers and when mosquitoes were being prepared for feeding by this method.

Some mosquitoes take up a very large amount of sugar water especially when confined in a small space, and many again will not feed very well. The best way of preparing mosquitoes for feeding is to put them on plain water only for a day or two and give them no fluid at all during the last 12 or 24 hours, keeping them at the same time at high temperatures so as to reduce the fat bodies if present. The difficulty experienced with mosquitoes kept in jars was that some would take up large amounts of sugar water, whereas others remained quite empty, and on starving them for some time, some would die before others would be ready for feeding.

Aëdes caballus is an exception to this rule as it will feed readily even when the abdomen is greatly distended with sugar water.

Later on large wooden cages, 36 inches long, 22 inches broad and 24 inches high, which were available at the laboratory, were used. These cages consisted of a framework of wood covered on three sides by mosquito netting, the one end being so constructed as to form a hinged door to facilitate cleaning or the placing of large objects inside the cage. The remaining side contained two glass panes, one on each side, to ensure good visibility from outside, and the centre panel, of mosquito netting, was provided with a thick wire ring, 4 inches in diameter, held in place by means of metal supports, to which was attached a sleeve of mosquito netting. Through this sleeve mosquitoes could be introduced or caught out of the cage with little chance of their escaping. The top and bottom of the cage were constructed of wood, overlapping the cage by about 1 inch. A sheet of galvanized iron, bent down at the sides, covered the top and a piece of hessian, of the same size as the galvanized iron top and having four flaps corresponding in size to the four sides of the cage, completely covered the cage. To the lower end of each hessian flap a piece of ¼ inch rod iron was sewn and these, when wedged in under nails driven horizontally into the edges of the floor of the cage, served to hold the hessian taut. Waste water was led away in a metal gutter attached to the bottom edge of the cage just below the lower ends of the hessian flaps.

To ensure a sufficiently high degree of humidity inside the cage the hessian cover was kept wet by running water on to the top of the cage. Water was supplied either direct from the mains or from an ordinary four gallon petrol tin standing on bricks on the roof of the cage and fitted on the underside with a gas tap. Once the hessian was wet a surprisingly small amount of water was needed to keep it so, and the contents of a petrol tin was more than enough to last out overnight.

Big balls of cotton wool, tied up in mosquito netting and soaked in a 10 per cent. solution of sugar water, were suspended from the roofs and supplied the mosquitoes with food. These balls had to be changed every second day on account of the growth of moulds, and were sterilized before being used again.

The results obtained by this method were quite satisfactory. The mortality amongst the mosquitoes was not excessively high and without special starving a large percentage was ready to feed, as could be noticed when introducing the hand into a cage. The only drawback was that different species of mosquitoes or different groups of the same species could not be separated. We finally adopted smaller specially constructed cages, consisting of a wooden framework, 21 inches long, 10 inches broad and 10 inches high, which could be

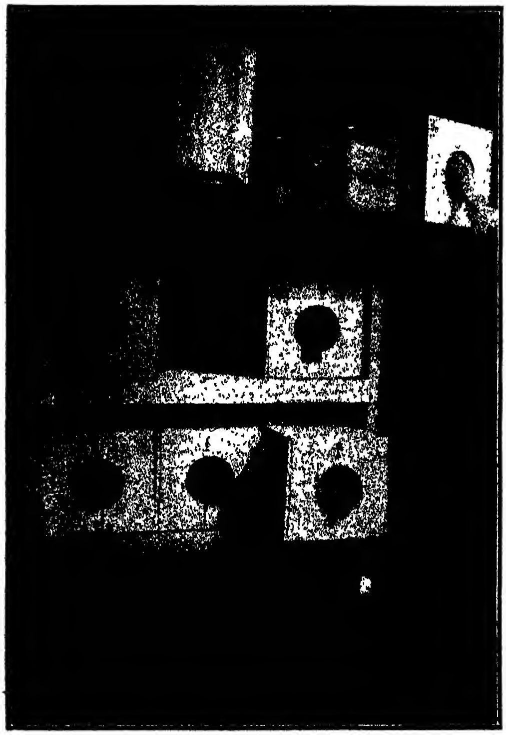

FIG. 1.

separated into two compartments, if necessary, by a sliding sheet of galvanized iron in the centre. They were covered on the two long sides and top with mosquito netting, the bottom and the two ends being made of wood. Each end contained a hole, large enough to admit the hand, and was fitted with a sleeve of netting. A framework, capable of holding 6 of these cages in two tiers of three, was constructed, and in order to ensure the desired degree of humidity inside the cages, the arrangement described above was used, viz., a metal top covered with hessian, having flaps hanging down on all sides, and a gutter for conducting away the waste water (c.f. fig. 1).

The results were practically as good as with the other methods and may be regarded as satisfactory. They were certainly not yet optimal and in the case of certain species better than with others. We do not yet know the reasons for some of the failures, but differences from the optimum humidity are in all probability the main factors. We had, however, not sufficient time and equipment at our disposal to go deeper into these problems.

With *Aëdes hirsutus* the results were very satisfactory indeed, only an insignificant mortality occurring. Only females, reared from larvae, were used. In one experiment, e.g. in March, 1932, 311 mosquitoes were fed on an infected horse, and after a period of 7 days 100 specimens were killed and injected into a horse, the others being used for feeding experiments. Of the remaining 211 specimens, 157 were alive 15 days after the initial feeding, 107 after 20-21 days, and 46 after 29-30 days. The conditions obtained in the cages were thus apparently quite favourable for *A. hirsutus*.

With *Aëdes lineatopennis* the results were also quite satisfactory. In one experiment carried out in March as well, for which mosquitoes reared from larvae and also specimens caught as adults were used, 151 specimens were fed on an infected horse. After a period of 19-21 days 71, or nearly 50 per cent., were alive, after 25-27 days 42, more than a month (33-35 days) after the initial feed, 38 specimens, and after an interval of as long as 60-62 days, 11 mosquitoes were still present. This result may certainly be regarded as good. In another experiment during the same month, 49 specimens out of 109 mosquitoes, or nearly 50 per cent., were still alive after a period of 16-18 days. In some of the other experiments the mortality was slightly higher. Even oviposition did not seem to affect this species very much, whereas in the case of *A. caballus*, the mortality, after the deposition of eggs, was considerable. On the whole we may say, that the conditions obtained in the cages were not unfavourable for *A. lineatopennis*.

Aëdes caballus was the most difficult species to keep alive for any length of time in captivity. When kept in the same manner as the other species the mortality was much higher. We endeavoured to still further raise the humidity by putting sheets of wet filter paper on the floors of the cages, but this did not help to decrease the mortality. In fact, the best results were obtained in our early experiments, when 10 specimens were stored in each of a number of ordinary jam jars. Adults caught in the field were more liable to die than those bred out from larvae in the laboratory, especially was this the case after oviposition, when the mortality was exceedingly high. As an illustration of the results obtained with this species, some experiments may be mentioned. In one group of experiments, 816 specimens were fed on an infected horse. Two weeks later only 100 specimens were left, and after a further week, not more than 10. In another set of experiments with a different strain of virus, out of 132 specimens used for injection into a horse, 68 mosquitoes, or about 50 per cent., were alive one week after the first feeding. Out of more than 900 specimens, which were used in feeding experiments, only about 10 per cent. survived 15-21 days. The highest mortality occurred as usual at the end of the first week after the initial feeding, when eggs were deposited. Although on this occasion most of the

groups consisted of mosquitoes reared from larvae, eggs were frequently laid. Females and males had hatched out together in the large cages, and before use had remained there together for some time, during which copulation must have taken place. The results were thus not ideal, but a certain number of specimens could be kept alive for the time required in our experiments. Furthermore the relatively high mortality was not too serious as large numbers of this species could be obtained by artificially flooding their breeding grounds.

Aëdes dentatus was more easily kept alive under laboratory conditions, however, not to the same extent as *A. hirsutus*. Out of 61 specimens, e.g., fed on an infected horse, 22 were still alive 25-27 days later, and 6 after 35-37 days. In another experiment, 129 mosquitoes engorged themselves on a horse and of these 63, or nearly 50 per cent., were still alive at the end of 14-21 days.

Lastly, *Aedes vittatus* was not difficult to keep alive in captivity, Our supply of this species was rather limited however.

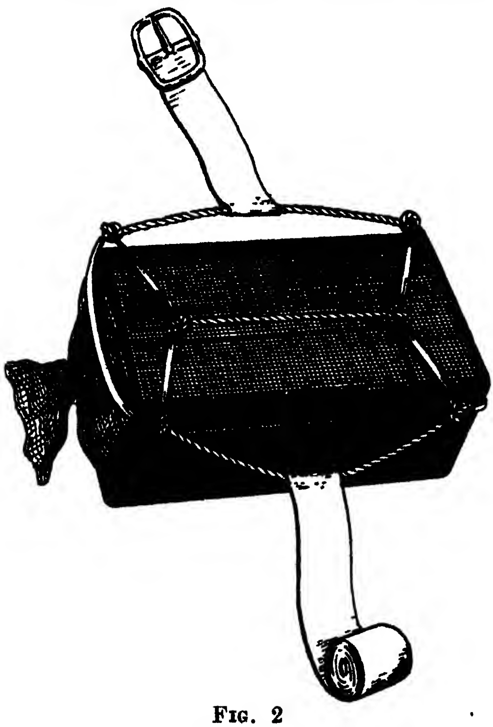

Fig. 2

3. METHODS OF FEEDING MOSQUITOES ON HORSES.

(a) Cages for Feeding Mosquitoes.

For feeding the mosquitoes on horses and sheep small cages, covered with mosquito netting, were used. These cages, containing the insects, were attached to the skin of the animals and were left there overnight. Cages of this sort had previously been used by one of us with good results in Europe for similar mosquito work (Nieschulz and Bos, 1931).

The cages were 5 inches long, 3 inches wide and 2½ inches deep, and consisted of a framework of strong wire, covered with a single layer of mosquito netting at the bottom and a double layer on the other sides (c.f. fig 2). A double layer of the ordinary mesh netting was necessary, otherwise small specimens could escape, especially at the edges. The netting projected at one of the small ends of the cage to form a sleeve 3-4 inches long through which the mosquitoes could be introduced and caught out later. The sleeve could be securely closed by tying it with tape. The measurements of the cages appeared to be optimal for our purposes. Larger cages were unsuitable as they did not fit flat on to the skin of the horse and left a space between the skin and the netting at the bottom of the cage which interfered with the feeding of the mosquitoes. Smaller cages would not have been capable of holding a sufficient number of mosquitoes. Our cages could quite easily accommodate 100-150 specimens without danger of their disturbing one another. For collecting the fed specimens from the cages, small test tubes, 6 inches long and ⅜-inch wide, were found to be most satisfactory on account of the small bore preventing the mosquitoes from leaving them easily and therefore as a rule remaining in them until the tube was removed from the cage and the opening plugged.

(b) Feeding Mosquitoes set Free in a Tent.

In a few experiments in February, 1932, the cages were not used. An infected horse was put into a box surrounded by a mosquito-proof tent standing in the veld near the laboratory, and a large number of newly hatched mosquitoes were set free in this tent. Quite a number of the insects engorged themselves, at any rate in two of the experiments, but in comparison with the numbers involved the results were by far not so good as with the cages. In the other experiment the result was not at all satisfactory.

(c) Arrangements for Fixing the Cages on the Horses.

The mosquitoes were fed on the backs of the horses, a patch of hair corresponding to the size of the cage being clipped short. Shaving was not necessary.

To start with we attached the cages to the horse by means of strips of ordinary plaster stuck to shaven areas around the clipped patch of skin. To the bottom edges of the cages a piece of calico about 1 inch broad was sewn, to which the plaster was fastened. The horse as put into a box which somewhat restricted its movements and each of the cages, attached to its back, was fastened to the ceiling of the stable by means of a piece of string which was sufficiently slack not to dislodge the cage should the horse move. If the animal fell down, e.g., when dying, the cages would be lifted off its back, hang on the strings, and thereby escape damage. This method worked quite satisfactorily and often over 90 per cent. of the mosquitoes were found engorged the next morning. However, difficulties were encountered when the horse was restless and reacted to the bites of the mosquitoes by twitching its skin. The insects were disturbed, which resulted in only a small number of specimens engorging themselves, or, it became loose before the mosquitoes could feed at all.

In trying to find a more suitable method of attaching the cages properly to the skin of the horse two strong rubber bands were put over the cage in the manner illustrated in fig. 2 to which two strips of strong linen (or leather straps) were attached. The one strip, which was long, went over the back and around the animal like a girth and was fastened to a short one on the other side of the cage. The rubber bands allowed for the normal movement of the animal's chest at the same time holding the cage in position and, in most cases at any rate, the results were quite satisfactory. This method was certainly an improvement but was still far from ideal.

Finally we had a special *saddle* constructed. It consisted of two galvanized sheet iron " saddle flaps " hinged on to a section of the same material, as shown in fig. 3. The saddle was lined by thick felt rivetted into position along the edges and each flap contained rectangular openings large enough for the feeding cages to fit easily. This saddle was held in position by means of an ordinary girth. The felt protected the animal from injury by the metal and at the same time tended to immobilize the skin when the horse was irritated by the bites of the mosquitoes. The cages containing the mosquitoes, after

FIG. 3.

being inserted into the rectangular holes of the saddle, were pressed down on to the skin by means of rubber bands which were fixed to hooks of which a pair was soldered on to the saddle flap above and below each opening. The pressure upon the cage could be varied by the number of rubber bands used and could be increased to such an extent that no movement could take place. In fact one had to be careful that the pressure was not too great as the cages often left the imprints of their frames on the skin, or even pressure necrosis resulted.

No special precautions were taken in cases where the horse fell or lay down, and this occasionally happened when the animal died overnight. The result, however, was that only the framework of some of the cages was bent, as only a portion of it projected outside, and the remainder was covered by the saddle which protected it to a certain extent. The mosquito netting covering the cages was never damaged and thus no mosquitoes could escape.

This method was very satisfactory in every case and may be regarded as the method of choice when working with horses. The mosquitoes are not disturbed at all, a large percentage engorge themselves, and the cages are very easily put into position on the horse and removed again.

(*d*) Feeding Mosquitoes in Ordinary Stables.

For the first experiments with horses, in September, 1931, only *Culex theileri* was available. The mosquitoes were fed in the small cages attached to the skin of the horse by strips of plaster. The horse itself was placed in a strong wooden box, just large enough to allow of some little movement, but preventing the animal from lying down. The box stood in an ordinary stable and a wide mosquito net was hung over the horse and box. Later on the use of this net was discontinued as it was found that no mosquitoes could escape from the cages. The experiments with *C. theileri* were concluded in October and the results obtained were satisfactory.

In November experiments with *Aëdes* species were commenced. The feeding results were not bad in November and the first part of December, but in some cases the percentage of engorged specimens was markedly less than was the case in the experiments with *Culex*, and a certain mortality, which occurred during the time the mosquitoes were on the horses, was also observed. This mortality later became very considerable, making experimental work almost impossible, as at times during January it rose to 90 per cent. The technique having remained the same, the changing climatic conditions alone could have been responsible.

(*e*) Influence of the Climatic Conditions on the Feeding Results.

In February, 1932, some experiments with mosquitoes set free in a fly proof tent were conducted. In the first experiment carried out on 1st February, during a wet night, the feeding results were good. The second experiment on 12th February was most unsatisfactory. Well over 1,000 specimens were liberated in this tent but only 95 engorged themselves on the horse and a very large number died without taking any food. It had been a dry day followed by a dry night and this failure strengthened our viewpoint that the prevailing low degree of humidity was responsible for the poor result. In order to verify this opinion we erected a similar tent near one of the buildings of the laboratory and fixed a hose above it in such a way that water could be run on to the roof and down the sides. The distribution was far from ideal, however, on account of the somewhat crude construction and, whereas a strong stream flowed down the one side, the other remained practically dry (fig. 4). The ground around the tent became thoroughly soaked and the splash of the falling water combined to create a fairly humid atmosphere. The mosquitoes were

again set free in the tent on 17th February and, notwithstanding the dry conditions which had prevailed during the day and night, the result was much better, almost 350 specimens engorging themselves on the horse. This constituted the final proof, so far as we were concerned, viz., that in order to obtain good feeding results, at any rate during the summer months, a humid atmosphere was an absolute essential.

(*f*) The Special Stable for Mosquito Feeding.

During the experiments which followed we used the same tent even when feeding mosquitoes in the small cages, and consistently good results, which were quite independent of outside weather conditions, were obtained. The tent was not an ideal place in which to work, however, and finally a special stable was designed and con-

Fig. 4.

structed, as shown in fig. 5. It consisted of a wooden framework 11 feet long, 8 feet wide and 7 feet 6 inches high. The roof was flat and consisted of a wooden framework covered by sheets of galvanized iron soldered together so as to render it water-tight. Mosquito netting covered the four sides, the lower edges of which were protected by flooring boards nailed to the uprights. The front side contained two doors, a large almost central one, measuring 6 feet 11 inches by 3 feet, through which the horse could be led in and removed, and a small door at one side, 33 by 34 inches, which gave us access to the stable. This small door was purposely made as small as possible so as to

minimize the chances of mosquitoes which had perhaps got out of one or other of the small cages, escaping. The whole stable was absolutely mosquito-proof and insects other than those used in the experiments were thus prevented from coming into contact with the horse at night. The importance of these precautions, especially in the case of susceptible horses, is self evident.

The whole stable, top and all four sides, was covered with hessian which could not come into contact with the mosquito netting on account of the roof overlapping the rest of the structure by 6 to 8 inches. In front the hessian was divided and hung down in the form

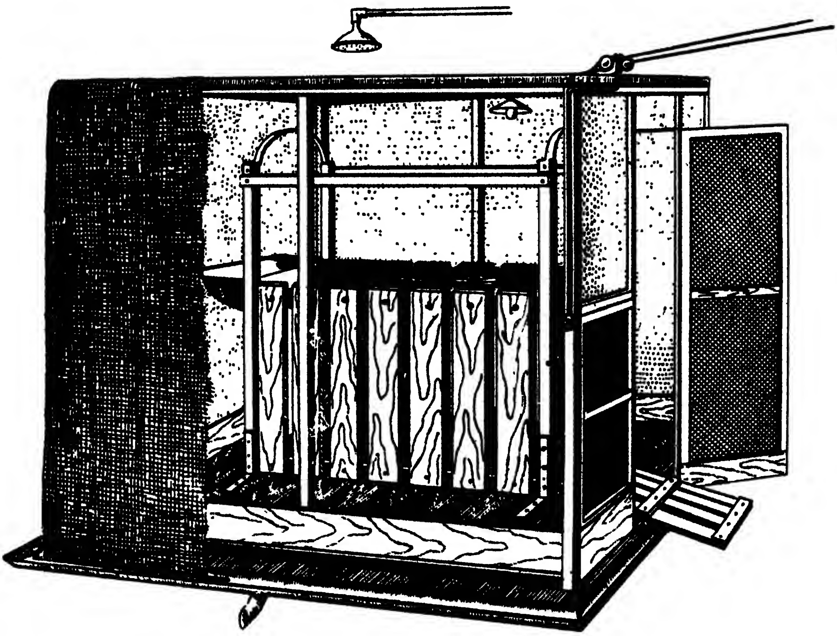

Fig. 5.

of curtains which could be lifted up so as to expose one or other of the doors. Along the lower edges of the hessian pieces of ½-inch rod iron were sewn which served to hold the hessian straight. Around the bottom of the stable galvanized guttering was attached into which the hessian hung, it being held taut by stout nails driven horizontally into the floor of the stable through the inner side of the guttering and under which the iron rods were forced.

Above the centre of the stable an ordinary bathroom shower was fixed but this was later replaced by a small garden spray, which spread the water more evenly and gave better results. On opening the water tap the hessian on top of the stable became soaked and the water was soon drawn evenly over all four sides by the hessian before being finally led away through the drain pipe in the guttering. The relative humidity within the stable rose to about 90 per cent. in a comparatively short time.

A strong box for the horse, 6 feet 6 inches long, 2 feet 6 inches wide, and only 3 feet 6 inches high, so as to render the back of the animal easily accessible, was firmly fixed to the floor near the centre of the stable. It was provided with a manger at the front end. Access to the box was provided for by the end opposite the manger being loose and hinged at the bottom so that it could be lowered and form a "walk in" to the box. This end was long enough to reach through the large door and over the guttering, which it protected from damage by the horses' hoofs, when lowered.

Between the sides of the box and those of the stable a working space of 3 feet on the one side and 2 feet on the other was left.

A fair amount of time was taken with the designing and construction of the stable with the result that it was only completed towards the end of March. From then on it was used continuously, combined with the saddle attachment for the small cages, and in the remainder of the experiments the feeding results were in all cases perfectly satisfactory.

(*g*) Times at which Experiments were carried out.

The mosquitoes were generally put on to the horses in the late afternoon, left on the animals overnight, and taken off again the following morning. Most of the mosquitoes used were night biting species and they had thus ample opportunity for feeding within their hours of predilection. From time to time, especially when large numbers had to be handled, they were also fed during the day-time. In most cases the results were not quite as good as during the night, but some species, especially *Aëdes caballus*, fed almost equally well during day-time.

(*h*) Feeding Results with Different Mosquito Species.

The different species responded differently to the opportunities for feeding offered them by means of the cage method. However, when suitable conditions were provided, every species could be induced to feed.

The easiest to feed was certainly *Aëdes caballus*. Quite regularly 90 per cent. were engorged in a relatively short time and they took up comparatively large amounts of blood. It was not even necessary to starve them beforehand as, often in cases where the abdomen was distended with sugar water, they still readily took up blood.

Aëdes hirsutus was almost as easy to feed as *A. caballus* and this species also took up a large amount of blood.

Aëdes lineatopennis occasionally presented difficulties, although on the whole the results could be regarded as satisfactory. The amount of blood taken up was generally less than in the case of the preceding species.

Aëdes vittatus also fed readily.

Culex theileri gave no difficulty at all and quite a large amount of blood was taken up by this species.

The few *Anophelines* used engorged themselves satisfactorily.

Mucidus scatophagoides was only tried towards the end of the season and, although previously regarded as a non-bloodsucking species, it was found to feed and take up large amounts of blood very readily.

4. METHODS OF FEEDING MOSQUITOES ON SHEEP.

For feeding the mosquitoes on sheep the small wire cages covered with mosquito netting, already described, were used in all cases. The liberation of mosquitoes in fly proof stables was not attempted, as no good results were expected.

To attach the cages to the sheep, the wool was carefully clipped, as close to the skin as possible, from an area on the back of the sheep corresponding to the one surface of the cage. The cage was then placed in position on this area and six pieces of tape, attached to the wool around the clipped area, two on each of the long sides and one on each of the short ends, tied together over it, served to hold it in position. In order to provide good contact and thereby facilitate the feeding of the mosquitoes, it is necessary that the tapes be pulled taut until the skin under the lock of wool to which each is attached is slightly lifted. Up to four cages can be placed on one sheep in this manner, two on either side of the mid line.

At first it was thought that conditions in these cages, which were in direct contact with the skin and surrounded on four sides by thick wool, would be suitable for the feeding of the mosquitoes, but it was soon found that, under our climatic conditions, the mortality amongst them was considerable, even though only placed on the sheep for a few hours at a time. Insufficient humidity was found to be the factor involved, and, in order to rectify this deficiency, a method, which proved both simple and effective, was devised. Before placing the cages in position the surface remote from the skin was covered with a piece of damp cotton wool which was held in position by the tapes passing over the cage. This had one obvious disadvantage of course, viz., that the mosquitoes could possibly take up water from the cotton wool, which was in close contact with the netting, and there is no doubt that in certain cases, where the mosquitoes failed to engorge, this did actually occur. In the greater majority of cases, however, the results were satisfactory.

As our stable for mosquito feeding, with special arrangements for obtaining a humid atmosphere, was mostly in use for horse-sickness experiments, the feeding on sheep had to be conducted in the ordinary sheep stables. On very dry nights the cotton wool occasionally dried up, resulting in a more or less high mortality amongst the mosquitoes, but under normal conditions, the water contained in the cotton wool was sufficient to last out the night.

By this method of mosquito feeding it was not necessary to isolate individual sheep as the cages are not interfered with by other sheep which may be present in the same box. Furthermore, the sheep used were apparently in no way irritated by the presence of the cage(s) as no attempt was made to rub it (or them) off, or, at any rate in our experiments, no sheep succeeded in dislodging a cage.

The feeding results on the whole were not so good as was the case in the horsesickness experiments. The percentage of mosquitoes which engorged themselves, and furthermore, the amount of blood taken up by the individuals was definitely less, whereas the mortality amongst the unfed specimens was certainly higher. A number of reasons may be advanced to account for this fact. The pressure exerted on the cage, on which depended its contact with the skin, was not so great as in the case of the cages used on horses, so that slight movements of the skin, tending to disturb feeding mosquitoes, could not be retarded to the same extent. The regulation of humidity was less effective, and as mentioned before, the mosquitoes had ample opportunity of taking up water. Short wool covering the clipped surface, which was never shaved, mechanically interfered with the insertion of the proboscis, and wool fat present probably also played a part in preventing good feeding.

However, our results were sufficiently satisfactory to permit of our disregarding these minor difficulties.

SUMMARY.

While engaged at Onderstepoort, during the winter of 1931 and summer of 1931-1932, in work on the transmission of horsesickness and bluetongue in sheep by means of mosquitoes, special methods, adapted to the South African climatic conditions, had to be devised.

The obtaining of a sufficiently high degree of humidity, to suit the requirements of the mosquitoes, was the most important factor to be contended with throughout the whole course of the work. The average humidity content of the air in Transvaal is so low that, especially during the summer months, the mosquitoes succumbed in considerable numbers, e.g., when left overnight in cages attached to a horse in one of the ordinary stables.

Larvae and adults were caught in the usual way. Adults of some of the species, e.g., *Aëdes caballus* and *A. lineatopennis*, could be caught from time to time in large numbers on their breeding grounds during the day.

The mosquitoes were kept in the laboratory in small jars or in cages enclosed by mosquito netting. To ensure a sufficiently high degree of humidity, the jars were placed on wet cotton wool in slightly larger jars provided with loosely fitting lids. The cages were kept on shelves in a wooden framework having a waterproof metal top covered above and on the sides by hessian kept wet constantly by a flow of water. By this means favourable humid conditions were provided within the cages in a manner similar to that employed in the larger mosquito cages previously described.

For feeding mosquitoes on infected or susceptible animals, small wire cages, covered by mosquito netting and firmly attached to a clipped area on the animal's skin, were used.

Of all the methods utilized, the best results, so far as attaching the cages to horses was concerned, were obtained by the use of a specially constructed metal saddle fixed to the horse in the usual manner. This saddle contained rectangular openings into which the cages just fitted, being held in position and firmly pressed against the skin of the horse by means of rubber bands.

For use on sheep the cages were held in position on the skin by means of pieces of tape attached to the wool around a clipped area in which these cages just fitted and tied together over each cage. A sufficiently high degree of humidity within the cages was obtained by placing damp cotton wool over each.

In order to obtain the humid conditions necessary for feeding mosquitoes on horses, a special stable was constructed consisting of a wooden framework with a metal roof, covered on the four sides by mosquito netting and having a further outer covering of hessian which was kept wet throughout the duration of each experiment by water distributed over the roof through a small garden spray. This stable contained a strong wooden box just large enough to accommodate the experimental horse, and guttering, for leading off waste water, together with electric lights, for night work, completed the equipment. The dimensions of the stable, viz., 11 feet by 8 feet by 7 feet 6 inches, were such as to allow of ample working space around the horse box. A sufficiently high degree of humidity, e.g., up to 90 per cent., could quite easily be maintained inside the stable.

With the use of the small mosquito cages held in position on horses by the special saddle under favourable conditions of humidity as provided for by the above-mentioned specially designed stable, and furthermore, feeding the mosquitoes at night, very satisfactory results were obtained.

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Echinococcus in Dogs from Pretoria and vicinity.

By R. J. ORTLEPP, M.A., Ph.D., Empire Marketing Board Research Officer, Onderstepoort, Transvaal.

THIS Institute is from time to time supplied by the Pretoria Municipal authorities with stray dogs which are caught in its boundaries. In this way the writer was able to examine and post-mortem 25 dogs, and the results of this survey is embodied in the following. It is difficult, if not impossible, to trace the exact origin of the dogs, but it may be taken, with little ground for doubt, that they have for the most part migrated into the municipal boundaries from the native locations situated in the vicinity of Pretoria. Except for three, all the dogs were infected with one or more helminths; eleven were infected with *Toxacara canis*, seven with *Toxascaris leonina*, five with *Ancylostoma caninum*, nineteen with *Dipylidium cananum* and five with *Echinococcus granulosus*. One dog was infected with all five. Of the five dogs which carried an *Echinococcus* infection, two harboured only a few worms, one had a fair infection and two had a heavy infection, the worms being located all the way down from the middle of the duodenum to near the caecum. Although these two dogs carried such a gross infection the intestinal mucosa appeared quite normal and it would thus appear that they suffered little, if any, inconvenience from their presence.

An examination of this material under the binocular microscope revealed the fact that the majority of worms had only three segments, the last of which was gravid. None were observed to have more, and a few had only two segments. This observation led the author to examine the material more carefully, especially as the European material from the English fox which he had examined while in London, had four or five segments, and material from the Hunting dog, *Lycaon pictus*, in the collection of this Institute, normally showed the presence of five segments, although some specimens with as many as seven segments were also present. This examination convinced the author that in South Africa at least two, and perhaps three, species of *Echinococcus* are present, all of which are different from the fox material which Cameron (1926) described as *E. granulosus*. The author identifies his material from the dog as *E. granulosus*, that from *Lycaon pictus* as a new species to which the specific name *lycaontis* is given, and he renames the material from the English fox giving it the specific name *cameroni*. The third South African species is probably *E. longimanubrium* which Cameron (1926) described from the Cape Hunting Dog, *Lycaon capensis*.

ECHINOCOCCUS GRANULOSUS (Batsch, 1786), Rud., 1805.

The material available for examination consisted of numerous specimens collected from the domestic dog. The specimens were collected alive and were killed and fixed in various ways; the most satisfactory method was to open out and place a portion of the intestine with the attached worms in slightly warmed tap water, and to leave them here until the worms were dead; this happened in

about two hours. The worms thus died beautifully extended and most of them detached themselves from the intestine; by carefully picking them up and dropping them into cold 10 per cent. formalin, they were found to undergo no shrinkage whatsoever. If, however, they were fixed in 70 per cent. alcohol they underwent considerable shrinkage, and the same also happened if the live worms were dropped into alcohol or formalin, hot or cold. The above method of killing and fixing, may not be satisfactory for histological studies, but for preserving the specimens in their natural condition it gave very satisfactory results; besides it was now possible to make out all the details of the genitalia and excretory systems by direct examination of the worms without any preliminary staining, and as the worms had died and been fixed in an extended position no flattening and consequent distortion of the specimens was necessary.

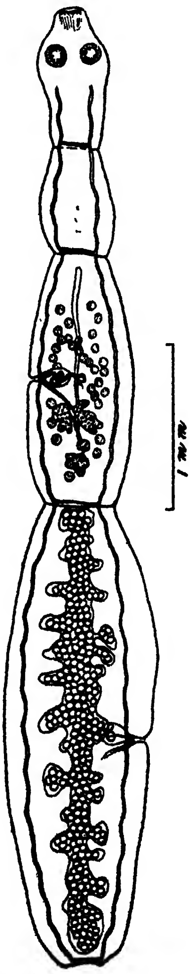

Fig. 1.—*Echinococcus Granulosus.*
Entire specimen killed in lukewarm water and fixed in 10 per cent. formalin.

Mature worms, carrying gravid segments, vary in length from 5 to 8·5 mm. with a maximum width across the gravid segment of 0·9 to 1·1 mm. Every worm carrying a gravid segment is built up of three segments plus a head and neck (Fig. 1), the first segment being immature and just showing the beginnings of the genital organs, the second segment contains the fully developed genital organs, and the last carries only the uterus with the contained eggs. Specimens with two segments have evidently lost their gravid segment as the second in these cases only contains the genitalia, or are still immature.

The head carries a prominent rostellum which is broader than it is long, varying in breadth from 0·16 to 0·25 mm. and from 0·1 to 0·2 mm. in length. At its apex it carries a crown of from 30 to 36 hooks arranged in two rows of 15 to 18 each. The hooks are fairly large, those of the anterior row being from 0·042 to 0·049 mm. in length, while those of the second row are 0·032 to 0·042 mm. long. The larger hooks have a stout blade placed at an angle to the axis of the handle and is strongly curved (Fig. 2). The guard is massive

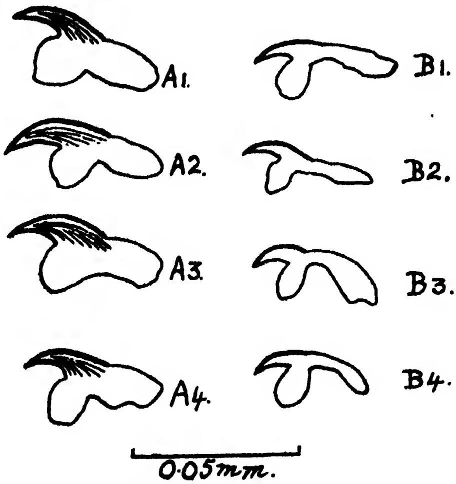

Fig. 2.—*Echinococcus granulosus* hooks.
A1—A4 hooks from anterior row.
B1—B4 hooks from posterior row.

and often has a cordate shape; the handle is also massive and generally thickens out towards its middle to taper towards its distal extremity; the guard and handle generally give the appearance of a bi-lobed base to the shaft, but a tri-lobed ventral outline is also seen in some of the hooks. Their edges are smooth. The smaller hooks are markedly different from those described above; the blade is smaller and lighter and is also less curved. The guard is relatively very massive and is somewhat oval in shape and its axis generally makes a more acute angle with the axis of the blade than is the case in the larger hooks. The handle is relatively very long and lies almost in a direct line with the axis of the blade. It may broaden out slightly towards its distal extremity, but in the majority of cases it maintains an even width and its edges may be slightly wavy; very

often its distal extremity is turned downwards. The suckers are round and prominent and vary in diameter from 0·16 to 0·2 mm. The head narrows immediately behind them to form the neck which is from 0·4 to 0·54 mm. long and 0·33 to 0·4 mm. in thickness.

The first segment is somewhat barrel-shaped and is slightly longer than broad; it varies in length from 0·38 to 0·75 mm. with a maximum thickness of 0·38 to 0·55 mm., it contains no definite organs, and the only indications of the genital organs is a median patch of darker cells in the posterior half of the segment.

The second segment varies in length from 1·1 to 1·8 mm. with a maximum breadth of 0·44 to 0·8 mm. It contains the mature genital organs; the number of testes varies from 30 to 53 and they are rounded with a diameter of 0·05 to 0·06 mm. They are situated in the middle portion of the segment, leaving its proximal and distal areas free. The majority are located on the aboral side anterior to the female genitalia. The vasa efferentia unite to eventually form a coiled vas deferens which enters the cirrus pouch at its distal extremity. The cirrus pouch is pyriform in shape and about 0·2 mm. long; it passes between the excretory canals and extends obliquely inwards and forwards, but does not reach the centres of the segment. The cirrus is retractile into the cirrus pouch and carries numerous rows of small spines. The genital pore lies at the centre of the segment and alternates irregularly. The vagina opens posterior of the cirrus pouch, the two forming a short common genital canal passing to the exterior. The ovary is roughly horse-shoe shaped, the limbs being however slightly lobed. In the isthmus connecting the two halves there is a distinct and spindle-shaped receptaculum seminis; from its anterior end the vagina passes obliquely forwards and outwards to the genital pore, while a thin duct passes backwards from it to terminate in the shell gland. This gland is roughly spherical in shape and is located slightly posterior of the ovaries. An irregularly-shaped but somewhat oval vitelline gland is present towards the posterior margin of the segment and its duct passes forwards to the shell gland where it meets the other ducts. The uterus also originates from the shell gland and this passes forwards in the centre of the segment as a slightly dilated tube.

The last or gravid segment only contains the uterus filled with eggs; it is relatively very large, being in most cases longer than the rest of the worm. It varies in length from 3 to 5·2 mm., with a maximum thickness of 0·8 to 1·1 mm. The uterus occupies most of its central area and is provided with about 12 to 15 lateral pouches. Most of its cavity is occupied by the numerous eggs. The embryophores are slightly oval with thick smooth and radially striated shells; they vary in size from 0·031 × 0·037 to 0·030 × 0·038 mm. and the hexacanth embryo varies in size from 0·021 × 0·026 to 0·022 × 0·028 mm., with hooks 0·01 mm. long. The wall of the empryophore is about 0·006 mm. thick. The excretory system is represented by two pairs of longitudinal ducts passing down each side of the worm; in the head region they become confluent with each other, and at the posterior end of each segment transverse ducts unite the corresponding ducts from each side.

DISCUSSION.

The most satisfactory description of this species available to the writer is that of Leuckart (1881). His description is based on material from dogs and differs in a few minor respects from the writer's material. He gives the maximum length of his specimens as 5 mm. whereas in the writer's material some of the specimens attained a maximum size of 8·5 mm. This difference in size may possibly be due to different methods of fixation and too great an importance can therefore not be assigned to this difference. Further, he says there may be three or four segments; in the writer's material no specimen was seen with more than three segments, but as Leuckart explains, it is quite possible that before the gravid segment is cast off an additional segment may in some cases have been formed, so that even if no four-segmented specimens were seen by the writer, the possibility of this number being present is not thereby excluded.

Leuckart states that the number of Rostellar hooks may vary from 28 to 50. The writer, however, has not seen such a wide variation in his material where the number varied only from 30 to 36. As to the sizes and shapes of the hooks in the two series, the writer's and Leuckart's findings agree very closely, the latter stating that the larger vary from 0·04 to 0·045 mm. and the smaller from 0·03 to 0.038 mm. as against the writer's 0·042 to 0·049 and 0·032 to 0·042 mm. respectively. Krabbe (1865), who also examined material from the dog, gives the number of hooks as from 38 to 40, and the variations in sizes as from 0·029 to 0·046 mm. for the larger and 0·021 to 0·033 for the smaller hooks; however, it is quite possible that the smallest hooks in both series may have belonged to immature individuals where the hooks had not yet grown to their full size. The writer also observed that the heads which carried the least number of hooks, tended to have their hooks larger than in those specimens with a larger number. Hall (1910) gives the sizes of the larger hooks as 0·022 to 0·03 mm. and those of the smaller as from 0·018 to 0·022 mm. Unfortunately he does not mention whether these measurements are based on his own observations, and if so, what the origin and nature of his material was, i.e. was it still immature or gravid? From Krabbe's, Leuckart's and Cameron's (1926) observations we know that the size of the hook (shaft and guard) increases in size with the development of the worm so that the size will depend to a great deal on the state of development of the parasite. Cameron, who based his observations on material from the English fox—*Vulpes vulpes*—gives the sizes of the larger and smaller hooks as 0·034 and 0·03 mm. respectively, but evidence will be brought forward later to show that Cameron did not have the true *E. granulosus* at his disposal, but a hitherto unidentified new species. Ross (1929) was able to examine numerous specimens of *E. granulosus* collected from the domestic dog in Australia. He found that the hooks varied considerably in size and shape, but he was not able to find any which corresponded closely with those figured by Cameron (1926). In his material the large hooks varied in length from 0·034 to 0·038 mm. and the small hooks from 0·022 to 0·03 mm. Although these hooks are smaller than those seen by the writer, there does not appear to be any reasonable grounds to doubt the identity of these materials. The most striking difference concerns the guard which is elongate and set almost at right angles to the axis of the handle

in Ross' specimens, whereas in the writer's it is less elongate, stouter and rounded, and its axis forms an obtuse angle with that of the handle. Krabbe (1865), however, figures both types, his specimens originating from Danish and Icelandish dogs. The hooks, however, agree in having a relatively long handle in that the guard is large, and in that the blade of the large hooks is strongly curved and the dimorphism between the two rows of hooks is marked. These characters are also well brought out in both Krabbe's and Leuckart's figures.

As far as the genitalia are concerned there is little difference between the writer's and Leuckart's findings. The latter finds about 60 testes present as against the writer's 30 to 53 and their size is slightly larger (0·07 mm.). The cirrus sac is smaller in the writer's material and does in consequence not extend to the middle of the segment as observed by Leuckart. Despite the above-mentioned differences the writer is satisfied that the materials examined by Krabbe, Leuckart, Ross and the writer all belong to the same species. In all four cases the materials originated from the domestic dog, that of Krabbe from Iceland and Danish dogs, in the former of which place *E. granulosus* is known to be an endemic parasite of dogs.

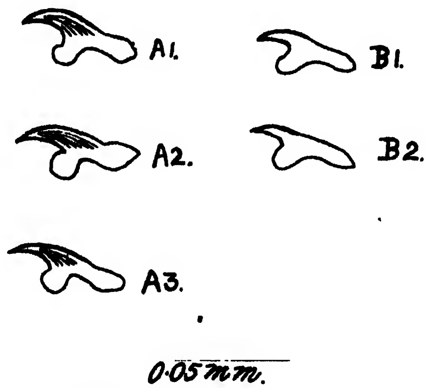

Fig. 3.—*Echinococcus cameroni* n. sp. hooks.
A1—A3 hooks from anterior row.
B1—B3 hooks from posterior row.

ECHINOCOCCUS CAMERONI n. sp.

SYN. *E. granulosus* OF CAMERON, 1926, FROM *Vulpes vulpes;* NOT *E. granulosus* (BATSCH) RUD., 1805.

In 1925 the writer collected adult *Echinococcus* from the common English fox—*Vulpes vulpes*—which had died in the Zoological Gardens in London. The writer identified this material as *E. granulosus*. Cameron (1926) reviewed the members of this genus and examined this same material and also concluded that it was *E. granulosus* and on this material he based his drawing and measurements of the hooks. Unfortunately, neither of us had an opportunity of carefully comparing this material with good material from the dog. The writer fortunately still has a few mounts of this fox material, and a comparison of these with the dog material described above has convinced the writer that the dog and fox materials are not co-specific. Cameron states that his fox material had two to

three immature segments (i.e. four or five segments), whereas some specimens from the dog which he examined had only three segments. In the writer's mounts there are also four or five segments, but none with only three segments, and the hooks are also similar to those described and figured by Cameron except that they are slightly larger, the larger being from 0·035 to 0·038 mm. long and the smaller from 0·03 to 0·033 mm. (Fig. 3). As the material examined by Cameron and in the writer's possession possesses gravid segments with ripe eggs, it appears legitimate to assume that the specimens are fully adult and that in consequence the hooks have attained their final form and size. A comparison of these with those of *E. granulosus* from the dog reveals the following differences:—

(1) The hooks are appreciably smaller; (2) the size dimorphism between the larger and the smaller hooks is much less marked than in *E. granulosus*; (3) the axis of the blade of the larger hooks tends to lie in a direct line with that of the handle, which is not the case in those of the dog material; (4) the size of the guard and sheath is relatively much more slender than in *E. granulosus*, where these structures are robust and are collectively much larger than the blade; (5) the handle of the smaller hook is relatively much shorter than that from the dog material.

From the above considerations of the size of the parasite, the number of segments, the size and shape of the hooks the writer concludes that this fox material represents a hitherto unidentified species which he has pleasure in naming after his friend and former colleague, Dr. T. W. M. Cameron. This species may briefly be diagnosed as follows:—

Slender parasites provided with four or five segments and from 5 to 7 mm. long. The mature segment is always the third from the end, the last being gravid, and the preceding being intermediate in development between the mature and gravid. The rostellum carries two rows of 28-32 hooks; those of the first row vary in size from 0·035 to 0·038 mm. and those of the second row from 0·03 to 0·033 mm. Size dimorphism between the larger and smaller hooks is not marked. The axis of the blade tends to lie in that of the handle. Other characters as for E. granulosus.

Location: Small intestine.
Locality: England.
Host: *Vulpes vulpes.*

In addition to the above two species, the writer has been able to examine representative material collected from the wild dog—*Lycaon pictus*, which had died in the Johannesburg Zoological Gardens. The material had been preserved in alcohol so that it is quite possible that the size given hereunder is appreciably less than their natural size. This species differs from the known species in several respects and in consequence it is here described as a new species.

ECHINOCOCCUS LYCAONTIS sp. n.

In general anatomical characters this species is similar to the genotype except in the number and nature of its hooks, and in the number of its segments. In consequence only those wherein it differs will be described.

The preserved material, provided with gravid segments, is 4 to 7 mm. long and the average number of segments is five (Fig. 4); however, specimens with four to seven segments are also present. In all forms the mature segment is the third from the last and the gravid the last; in no case is there more than one mature and one gravid segment present. The most remarkable character of this parasite is the presence of four rows of hooks on the rostellum; the

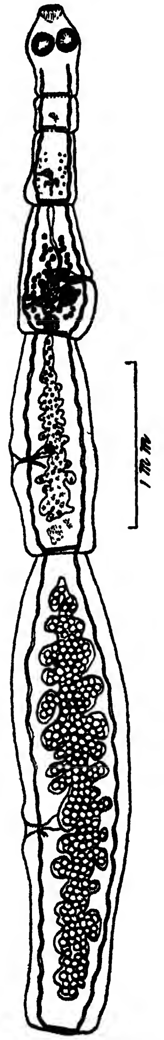

Fig. 4.—*Echinococcus Lycaontis* n. sp.
Entire specimen killed and fixed in alcohol.

first and second rows conform in general with those typical for the genus, but those of the third and fourth rows are very much smaller, and appear to drop off much more easily than those of the anterior two rows (Fig. 5). No scolex was seen on which the full number of these small hooks were still present, notwithstanding that all those

of the first and second rows were still present. Each of the anterior two rows carries 17 or 18 hooks, which are slightly smaller than those of *E. granulosus*. The larger hooks are from 0·04 to 0·045 mm. long and the smaller 0·028 to 0·033 mm. The former carry a stout blade which is not as strongly arched as that of the dog material, and the shaft (handle and guard) is also more slender. The axis of the blade meets that of the shaft at an angle, and there is, in most cases, a distinct notch on the dorsal surface where the blade meets the handle. The ventral margin of the shaft is seldom saddle-shaped, as is often the case in *E. granulosus*, but tends to have a tri-lobed outline. The guard is relatively small and is either rounded, oval or cordate in shape. The handle is plump and its distal extremity is either rounded or tapering. The hooks of the second row are much more slender than those of the first row. The blade is less arched,

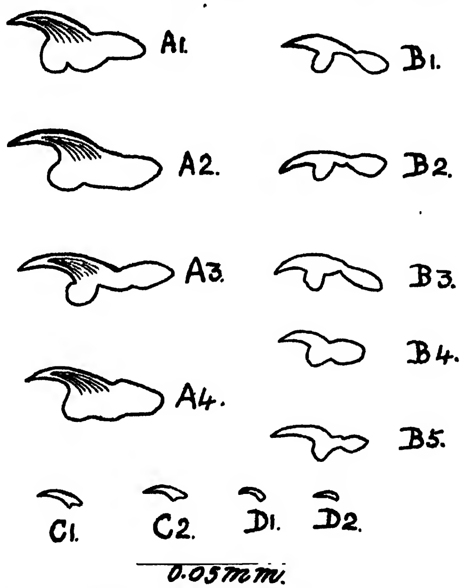

Fig. 5.—*Echinococcus lycaontis* n. sp. hooks.
A1—A4 hooks from anterior row.
B1—B5 hooks from second row.
C1—C2 hooks from third row.
D1—D2 hooks from fourth row.

the guard in general is oval in shape and the handle generally terminates in an oval swelling. This last character serves to distinguish these hooks easily from the corresponding hooks of *E. granulosus* or *E. cameroni*. The hooks of the third and fourth rows are very small, and are composed of only the blade, the absence of a shaft probably gives the reason why they are so easily lost. Those of the third row appear to be lodged opposite those of the first row, and those of the fourth row opposite those of the second row. It was not possible to determine their exact number as they were incomplete in all the specimens examined. The larger of these hooks are from 0·012 to 0·014 mm. long and the smaller from 0·005 to 0·006 mm. Their blades are only slightly arched. In the smaller hooks there is no indication whatever of a guard or handle, whereas those structures are represented in the larger hooks by two short processes at the

distal extremity of the blade. The neck is narrower than the rest of the head and leads to the first segment which is slightly longer than broad and shows the beginnings of the genitalia as a streak of darker staining cells in the middle of the posterior half of the segment. In specimens with five segments the second segment is about half as long as it is broad; in it the testes, ovaries and yolk gland and their ducts have become differentiated. The third segment in six and seven segmented forms is about half again as long as the second segments, and in it the genital organs are slightly better developed although not mature; in five-segmented forms the genital organs are fully mature in this segment; the details of this system are similar to those described by *E. granulosus* above, except that, on account of shrinkage (?), the individual organs are smaller. The fourth, or fifth, or sixth segments in five, six and seven segmented worms respectively show the disappearance of the genital organs and the enlargement of the uterus which becomes filled up with the maturing eggs. These segments are from two to three times as long as they are broad. The last segments are gravid and may form from a half to three-fifths of the entire length of the parasite, and is nearly five times as long as it is broad. The uterus, with about a dozen sacculations on each side, occupies the greater portion of its central area and is practically filled with embryophores indistinguishable from those of *E. granulosus.* The genital pores alternate irregularly and are situated just posterior of the middle of each segment.

Host: *Lycaon pictus.*
Location: Small intestine.
Locality: Johannesburg Zoological Gardens.

DISCUSSION.

The presence of four rows of hooks on the rostellum places this species in a unique position among the remaining members of the genus *Echinococcus.* A careful search has been made for them in the specimens from the domestic dog and English fox, but in not a single case was there the faintest trace of them seen. It may thus be taken with confidence that this characteristic is specific for this parasite. The number of segments is also peculiar to this species, there being, as far as the writer is aware, no previous record of an *Echinococcus* possessing up to seven segments. The size and shape of the hooks on the first and second rows is also different from those known in described species. Taking these three characters together there does not appear to be any doubt that this material represents a hitherto unknown species.

Specific Diagnosis.

Slender parasites provided with four to seven segments and 4 *to* 7 *mm. long in specimens preserved in alcohol. The mature segment always third from last. Rostellum carries four rows of hooks; the shapes and sizes of those of the first and second rows conform to those typical of genus and vary in number from* 34 *to* 36 *and in size from* 0·04 *to* 0·045 *mm. for the larger and* 0·028 *to* 0·033 *mm. for the smaller; hooks of third and fourth rows small, degenerate and easily lost, their handles and guards being entirely absent or only represented by short processes. Internal morphological characters as for* E. granulosus.

Cameron (1926) described from a nearly related host, the Cape hunting dog, *Lycaon capensis*, a new species which he named *E. longimanubris*. He was unfortunately not able to give a detailed description, because the material was not sufficiently well preserved. He, however, gives measurements and drawings of the hooks and further mentions that " in general appearance it closely resembles the ordinary species from the dog ". As the dog specimens which Cameron saw had three segments, the writer concludes that this species also had only three segments. This character would thus serve to differentiate his species from the species described above. In addition there are considerable differences in the hooks, the larger hooks are smaller, 0·035 mm. as against 0·04 to 0·045 mm. the guard is remarkably large and situated at right angles to the axis of the blade and handle, and the handle of the smaller hooks are much larger and do not end in expanded extremities.

Principal Measurements in mm. of the Six Known Species of Echinococcus.

	E. granulosus.	*E. cameroni.*	*E. lycaontis.*	*E. oligarthrus.*	*E. longimanubris.*	*E. minus.*
Size..........mm.	5–8	5–7	4–7	1·7–2·5	?	?
No. of segments..	3–4	4–5	5–7	3	?	?
No. of hooks......	30–36	28–32	68–72 (34–36 large)	36–40	?	?
Size of large hooks......mm.	·042–·049	·035–·038	·040–·045	·045	·035	·032
Size of small hooks......mm.	·032–·042	·030–·033	·028–·032	·032	·030	·020
No. of testes......	33–53	50–60	40–50	20–33	?	?

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Section III.

Poisonous Plants.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 3, Number 1, July, 1934.

Isolation of the Poisonous Principle of *Dimorphotheca cuneata* Less.

By

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IN a recent report from this Laboratory, the isolation from *Dimorphotheca spectabilis* Schltr. and *Dimorphotheca Zeyheri*, Sond. was described (Rimington, 1932) of the cyanogenetic glucoside which renders these plants poisonous to stock. The active principle was identified with acetonecyanhydrin glucose ether or "linamarin", first obtained from *Linum usitatissimum*.

In the present instance, *Dimorphotheca cuneata*, Less. has been studied and the same glucoside shown to be present.

Dimorphotheca cuneata grows fairly profusely in certain districts in the vicinity of Grahamstown and has been recognized by Curson (see Steyn, 1932) as one of the plants causing death from prussic acid poisoning (geilsiekte) in those areas. No other reference to its toxicity could be found.

COLLECTION OF MATERIAL.

The material used in the present investigation was obtained, through the kindness of the Principal Botanist of the Division of Plant Industry, from plants growing in the grounds surrounding the National Herbarium, Pretoria. Reference specimens were deposited, Nos. 15720, 15725.

Determinations of the total hydrogen cyanide yielded by the various parts of the plant were made as previously described. In the case of *Dimorphotheca cuneata*, it was found advisable to allow maceration to proceed for 36 hours since enzymatic hydrolysis of the glucoside is not so rapid as in the other two species which have been studied. For comparison, analyses were also made of plants growing in the Onderstepoort poison garden. The results are recorded below:

Material from National Herbarium Grounds.

	Moisture percentage.	*Mgm. HCN per 100 gm. of fresh material*	*Mgm. HCN per 100 gm. calculated dry weight.*
Whole plant	55·7	124·8	281·7
Green stems	37·6	74·3	119·0
Leaves	58·3	164·9	399·5
Flowers	67·6	46·8	144·5
Seeds	61·1	24·5	63·0

Material from Onderstepoort Poison Garden Gathered in the Early Morning (Onderstepoort Herbarium No. 7411).

	Moisture percentage.	Mgm. HCN per 100 gm. of fresh material	Mgm. HCN per 100 gm. calculated dry weight.
Whole plant	66·2	17·1	50·6
Green stems	44·9	8·7	15·7
Leaves	64·2	47·1	131·4
Flowers	70·7	46·2	157·8
Seeds	66·7	19·3	57·9

Specimens of the same Material Gathered at noon on a Hot Day.

	Moisture percentage.	Mgm. HCN per 100 gm. of fresh material.	Mgm. HCN per 100 gm. calculated dry weight.
Stems	40·0	13·4	22·3
Leaves	63·0	130·3	352·2
Roots	37·0	183·5	291·4

The above results would suggest a diurnal variation in hydrogen cyanide content and possibly a correlation with intense photosynthetic activity. These and other physiological aspects of cyanogenesis it is hoped to study more closely at a later date.

Proximal analysis of the material in the seeding stage afforded the following figures:—

Moisture content of plant powder	4·2	per cent.
Crude protein (on dry weight basis) ...	11·6	per cent.
Ash	7·3	per cent.

Determination of Acetone.

Determinations of the quantity of acetone liberated simultaneously with hydrogen cyanide when the plant is macerated in buffer solution were carried out as follows, since it was deemed of interest to ascertain whether this reaction could also be used for the quantitative determination of glucoside.

100 gm. of plant powder (post-flowering stage) was allowed to macerate for 36 hours, excess of a suspension of lead hydroxide was then added and the mixture steam distilled, the receiving flask being surrounded by ice. The distillate was treated with a slight excess of silver nitrate solution, the volume measured and the mixture filtered. To an aliquot (approximately 60 mgm. acetone), 50 c.c. of N sodium hydroxide followed by 100 c.c. N/10 iodine solution were then added and the mixture shaken for 10 minutes. The acetone is converted into iodoform. 50 c.c. of normal sulphuric acid was added and the excess of iodine titrated by N/10 sodium thiosulphate.

Acetone found = 0·1606 gm. per 100 gm. plant powder.
calculated from

HCN determination = 0·1433 gm. per 100 gm. plant powder.

The presence of acetone in the steam distillate may render a strictly quantitative determination of hydrogen cyanide unrealisable by the Liebig titration method, on the other hand it can not be assumed that all of the iodoform-forming material in the above experiment was derived from hydrolysed glucoside.

It will be observed that in the Onderstepoort material the hydrogen cyanide content of the stems was very low, which is possibly to be correlated with the fact that these plants were stunted and very woody whilst those grown in Pretoria were much more luxuriantly developed. The flowers and seeds, however, contained approximately the same amount of hydrogen cyanide in the two cases. Compared with *Dimorphotheca spectabilis*, the yield of hydrogen cyanide from *Dimorphotheca cuneata* is only about one-tenth as great.

Isolation of the Toxic Principle.

The plants were air-dried for some days and then reduced to powder in a mill. Determinations of hydrogen cyanide showed that considerable loss of glucoside had occurred, the powder containing:

	Free HCN mgm. per 100 gm.	*Total HCN mgm. per 100 gm.*
Material in flowering stage	11·1	144·7
Material in post-flowering stage	13·4	66·7

It should be mentioned that the leaves and stems of *Dimorphotheca cuneata* contain a fairly large quantity of a resinous substance which not only retards the drying of the plants but also renders the determination of the hydrogen cyanide content more difficult than in the other species. Volatile constituents of the resin pass over into the steam distillate rendering the solution slightly turbid and thus tending to obscure the end point of the titration. A gradual increase in the weight of the oven-dried material was observed and would suggest the possible oxidation of resinous constituents by atmospheric oxygen. Plants growing in sandy soils were not easily freed from grit adhering to the stems and leaves. 175 gm. of the plant powder was dropped into 1 litre of boiling water containing a little calcium carbonate. After some hours the liquid was pressed off and the plant residue again extracted. The combined extracts were treated with lead acetate, the precipitate filtered off, washed and discarded, and excess of lead removed by hydrogen sulphide. After aerating the filtrate, sodium hydroxide was added to neutrality, and the liquid concentrated under reduced pressure to the consistency of a thick syrup. 96 per cent. alcohol was then stirred in, two volumes of ether added and the resulting precipitate discarded. The filtrate was concentrated, mixed with a fairly large quantity of decolorising charcoal and dried. Water was then added, the mixture filtered and the filtrate again evaporated to dryness. This residue was taken up in 96 per cent. alcohol, two volumes of ether added and the filtrate again concentrated to dryness. The material remaining, which was still of a syrupy consistency was extracted repeatedly with boiling anhydrous ethyl acetate. The combined extracts were concentrated on the water bath to dryness and the residue, after washing with ether, re-extracted with fresh portions of boiling ethyl acetate. This procedure was repeated until no material insoluble in ethyl acetate remained. Since the ethyl acetate solution still had a yellowish colour and contained resinous material which interfered with crystallisation, the evaporated extract was taken up in a little water, boiled with decolorising charcoal until colourless and then evaporated to dryness, the last

traces of water being removed in a vacuum desiccator. The material was recrystallized until pure from boiling ethyl acetate. Yield 12 mgm. The glucoside so obtained had all the properties of "linamarin". It melted at 139-140°. The optical rotatory power* was determined in a 2 dm. tube using sodium light.

$$[\alpha]_D^{27} = \frac{-0\cdot738 \times 14}{2 \times 0\cdot2023}$$
$$= -25\cdot5°.$$

For synthetic linamarin Fischer and Anger (1919) give M.P. 141-2° and $[\alpha] = -29\cdot1°$. Upon micro-analysis it yielded the following figures per cent.:—

	C	H	N
	48·20	7·17	5·72
$C_{10}H_{17}O_6N$ requires	48·54	6·93	5·67

The substance is identical, therefore, with acetonecyanhydrin-glucose ether.

The tetra-acetyl derivative was prepared by acetylation at room temperature with acetic anhydride-pyridine mixture. It crystallised in needles M.P. 139°. Fischer and Anger give M.P. 140-141°.

Direct Ethyl Acetate Extraction Method.

Since the isolation of the glucoside by the methods already described was attended by considerable losses, especially in the present case of *Dimorphotheca cuneata*, a plant containing much more resinous material than does either of the other two species investigated, an attempt was made to extract the glucoside directly from the dried, powdered plant by means of boiling ethyl acetate.

For this purpose, 300 gm. of the plant was used, three large soxhlet extractors being employed and the extraction continued for 24 hours. The bulk of the solvent was distilled off, the extracts combined and evaporated to dryness upon the water bath followed by exsiccation. The dark tarry residue was thorouglhy extracted with boiling water, the aqueous solution decolorised by means of charcoal and then evaporated to a syrup which was dried in a vacuum desiccator. The material remaining was extracted exhaustively with boiling ethyl acetate, the extract evaporated and dried, then again taken up in boiling ethyl acetate, this procedure being repeated until the glucoside crystallised out in pure form on cooling the hot liquid. Yield 0·5 gm. M.P. 139-140°. The yield represents only about 14 per cent. of the glucoside actually present in the plant material as shown by HCN determinations, but is superior to that obtained by the water extraction method and the whole procedure is much less laborious. Using a specimen of *Dimorphotheca spectabilis*, a yield of 88·5 per cent. of pure glucoside was easily attained.

The Nature of the Enzyme Present in the Plant.

A preparation of the enzyme present in the plant and normally causing the decomposition of the glucoside was made as follows:—

* Determined upon a sample subsequently isolated by the direct ethyl acetate method.

40 gm of plant powder were extracted with cold water and the solution centrifuged Five volumes of 96 per cent alcohol were then added and the flocculent precipitate collected on the centrifuge, washed with ether and dried rapidly in a vacuum desiccator over sulphuric acid. The powder so obtained was almost completely soluble in water, the residue being centrifuged off before use Solutions were prepared of linamarin, amygdalin and salicin, enzyme added and these, together with suitable controls, incubated at 37°.

Dimorphotheca cuneata Less

The results were as follows:—

Enzyme plus	linamarin	amygdalin	salicin
	+ + rapid action	slowly +	–

Its action upon α-methylglucoside was investigated by incubating solutions of enzyme and glucoside for 7 days at 37° (in the presence of a trace of chloroform), then adding basic lead acetate, centrifuging and comparing the optical rotatory powers of the filtrates.

	Rotation observed.
Enzyme + α-methylglucoside	+1·41
Enzyme + α-methylglucoside, boiled	+1·43
Enzyme + water	0

It appears that, as in the case of the other two species investigated, *Dimorphotheca cuneata* contains an enzyme specifically adapted to its substrate and having but little action upon other β-glucosides and none upon α-methyl glucoside. The name "linamarase" has been suggested for this enzyme (Rosenthaler, 1922; Rimington, 1932). Liberation of HCN from the plant is slower than in the case of the other two *Dimorphotheca* species. An aqueous extract of dried yeast, after purification by alcohol precipitation, was still found to liberate HCN from linamarin.

The Toxicity of the Plant and Determination of the M.L.D.

Feeding tests upon rabbits were carried out using the ground, dried material which was suspended in water and dosed by stomach-tube. The M.L.D. was found to be between 14 and 20 gm. (seeding stage) for approximately 2 Kilo rabbits corresponding to ±14 mgm. HCN per Kilo body weight. The toxic action appeared to be much slower than in the case of *Dimorphotheca spectabilis* and *Dimorphotheca Zeyheri* poisoning, a fact to be correlated, possibly with the more feeble enzymic activity of *Dimorphotheca cuneata*.

As in the case of other *Dimorphotheca* species, it is to be expected that the prophylactic measure advocated by Steyn (1929) of dosing sulphur, incorporated in a lick, should also serve to protect stock animals from the poisonous effects of *Dimorphotheca cuneata*.

Summary.

The toxic substance present in the plant *Dimorphotheca cuneata* has been isolated and identified as the cyanogenetic glucoside "linamarin" or acetonecyanhydrin glucose ether. An improved method of extraction has been described.

The plant contains the enzyme "linamarase" exhibiting a fairly high degree of specificity for its substrate.

Quantitative determination shows that *Dimorphotheca cuneata* in the flowering stage yields about 280 mgm. of HCN per 100 gm. plant, calculated on the dry weight basis. The toxic effects following ingestion of lethal quantities of the plant appear to develop more slowly than is usually the case with *Dimorphotheca* species.

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Plant Poisoning in Stock and the Development of Tolerance.

By

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(Continued from *Onderstepoort Jour. Vet. Sci. and Animal Ind.*, Vol 1, 1933, pp. 149-156.)

GIFBLAAR [*Dichapetalum cymosum* (HOOK.) ENGL.]

In previous publications (Steyn, 1932 and 1933) it was mentioned that animals develop a tolerance to *Chrysocoma tenuifolia* Berg and *Centaurea picris* D. C., whilst this was not the case with *Asclepias physocarpa* Schltr.

Field observations seem to indicate that gifblaar does not belong to those plants which induce the development of tolerance when eaten in non-lethal quantities over prolonged periods.

In the tables given below the results of two experiments are recorded:—

Tolerance Experiment.—Gifblaar Leaves.

Rabbit No.	Wgt. in Kg.	Amount of Plant Given per Kg. Body-weight.	Result.
A	2·8	0·05 gm. dry leaves from 24/4/33–29/4/33 0·1 gm. dry leaves from 1/5/33– 5/5/33 0·2 gm. dry leaves from 11/5/33–12/5/33 Total amount of leaves received = 3·36 gm.	Died suddenly on 13/5/33 from gifblaar poisoning.
B	2·7	0·05 gm. dry leaves from 24/4/33–29/4/33 0·1 gm. dry leaves from 1/5/33– 5/5/33 0·2 gm. dry leaves from 11/5/33–12/5/33 Total amount of leaves received = 3·24 gm.	Tolerance test on 18/5/33: Received 1·0 gm. dry leaves per Kg. body-weight. Died from gifblaar poisoning 12 hours after having been drenched.
C	2·3	0·05 gm. dry leaves from 24/4/33–29/4/33 0·1 gm. dry leaves from 1/5/33– 3/5/33 Total amount of leaves received = 1·38 gm.	Died suddenly on 3/5/33 from gifblaar poisoning.
D	1·9	0·05 gm. dry leaves from 24/4/33–29/4/33 0·1 gm. dry leaves from 1/5/33– 5/5/33 0·2 gm. dry leaves from 11/5/33–12/5/33 Total amount of leaves received = 2·3 gm.	Tolerance test on 18/5/33: Received 1·0 gm. dry leaves per Kg. body-weight. Died from gifblaar poisoning 10 hours after having been drenched.
E	1·9	Treated as D.......................... Total amount of leaves received = 2·3 gm.	Tolerance test on 18/5/33: Received 1·0 gm. dry leaves per Kg. body-weight. Died from gifblaar poisoning 6 hours after having been drenched.

Rabbit No.	Wgt. in Kg.	Amount of Plant Given per Kg. Body-weight.	Result.
F	1·5	Treated as D.......................... Total amount of leaves received = 1·8 gm.	Tolerance test on 18/5/33: Received 1·0 gm. dry leaves per Kg. body-weight. Died from gifblaar poisoning 5 hours after having been drenched.
G	2·1	Treated as D.......................... Total amount of leaves received = 2·52 gm.	Tolerance test on 18/5/33: Received 1·0 gm. dry leaves per Kg. body-weight. Died from gifblaar poisoning 12 hours after having been drenched.
H	1·8	Treated as D.......................... Total amount of leaves received = 2·16 gm.	Tolerance test on 18/5/33: Received 1·0 gm. dry leaves per Kg. body-weight. Died from gifblaar poisoning 12 hours after having been drenched.
I	1·8	Treated as D.......................... Total amount of leaves received = 2·16 gm.	Tolerance test on 18/5/33: Received 1·0 gm. dry leaves per Kg. body-weight. Died from gifblaar poisoning 16 hours after having been drenched.
J	2·4	0·1 gm. dry leaves from 24/4/33–29/4/33 0·2 gm. dry leaves from 1/5/33– 5/5/33 0·4 gm. dry leaves from 11/5/33–12/5/33 Total amount of leaves received = 5·56 gm.	Tolerance test on 18/5/33: Received 1·0 gm. dry leaves per Kg. body-weight. Died from gifblaar poisoning 14 hours after having been drenched.
K	1·8	0·1 gm. dry leaves from 24/4/33–29/4/33 0·2 gm. dry leaves on 1/5/33 Total amount of leaves received = 1·44 gm.	Died suddenly on 1/5/33 from gifblaar poisoning.
L	2·1	0·1 gm. dry leaves from 24/4/33–29/4/33 0·2 gm. dry leaves from 1/5/33– 2/5/33 Total amount of leaves received = 2·1 gm.	Died suddenly on 2/5/33 from gifblaar poisoning.
M	1·7	Treated as J.......................... Total amount of leaves received = 4·08 gm.	Tolerance test on 18/5/33: Received 1·0 gm. dry leaves per Kg. body-weight. Died from gifblaar poisoning 8 hours after having been drenched.
N	1·9	0·1 gm. dry leaves from 24/4/33–29/4/33 0·2 gm. dry leaves on 1/5/33 Total amount of leaves received = 1·52 gm.	Died suddenly on 1/5/33 from gifblaar poisoning.
O	2·1	Treated as J.......................... Total amount of leaves received = 5·04 gm.	Tolerance test on 18/5/33: Received 1·0 gm. dry leaves per Kg. body-weight. Died from gifblaar poisoning 5 hours after having been drenched.
P	2·0	0·1 gm. dry leaves from 24/4/33–29/4/33 0·2 gm. dry leaves from 1/5/33– 3/5/33 Total amount of leaves received = 2·4 gm.	Died suddenly on 3/5/33 from gifblaar poisoning.
Q	2·2	0·1 gm. dry leaves from 24/4/33–29/4/33 0·2 gm. dry leaves on 1/5/33 Total amount of leaves received = 1·76 gm.	Died suddenly on 1/5/33 from gifblaar poisoning.
R	2·3	0·1 gm. dry leaves from 24/4/33–28/4/33 Total amount of leaves received = 1·15 gm.	Died suddenly on 28/4/33 from gifblaar poisoning.

The gifblaar leaves used in the above experiment were collected in a very young stage of development on the Magaliesberg, Pretoria North. The leaves were air-dried, and ground, and the powder thoroughly mixed before use. The M.L.D. per Kg. body-weight rabbit was found to be 0·75 gm.

It was intended to administer the dry leaves as follows:—

A. *Nine Rabbits (A to I).*

0·05 gm. (i.e. 1/15 M.L.D.) dry leaves per Kg. body-weight from 24.4.33 to 29.4.33 (inclusive).

0·1 gm. (i.e. 2/15 M.L.D.) dry leaves per Kg. body-weight from 1.5.33 tot 5.5.33 (inclusive).

0·2 gm. (i.e. 4/15 M.L.D.) dry leaves per Kg. body-weight from 11.5.33 to 12.5.33 (inclusive).

B. *Nine Rabbits (J to R).*

0·1 gm. (i.e. 2/15 M.L.D.) dry leaves per Kg. body-weight from 24.4.33 to 29.4.33 (inclusive).

0·2 gm. (i.e. 4/15 M.L.D.) dry leaves per Kg. body-weight from 1.5.33 to 5.5.33 (inclusive).

0·4 gm. (i.e. 8/15 M.L.D.) dry leaves per Kg. body-weight from 11.5.33 to 12.5.33 (inclusive).

This manner of dosing was adopted as previous experiments upon rabbits seemed to indicate that the plant has cumulative effects, i.e., that the active principle is inactivated or excreted very slowly.

Of the nine rabbits in group A and group B two and six died respectively before the tolerance test could be applied. The remaining ten rabbits received 1·0 gm. (i.e. 1⅓ M.L.D.) dry gifblaar leaves per Kg. body-weight on the sixth day after the preliminary treatment with small quantities of dry leaves had been discontinued. Not a single animal survived the tolerance test, thus clearly indicating that no decrease in susceptibility to gifblaar had occurred in the course of the preliminary treatment. It is hardly possible that an increase in resistance had occurred but disappeared in the interval that elapsed between the time of discontinuation of the preliminary treatment and the application of the tolerance test.

The results recorded in the above table clearly indicate the difference in susceptibility of the different animals to gifblaar. They also bring out the fact that the active principle of gifblaar is inactivated in, or (and), excreted from the body at a very slow rate. The M.L.D. of the dry leaves per Kg. body-weight of rabbit was determined as 0·75 gm., and yet we find the following noteworthy cases in the above table: (*a*) *Rabbit C* (2·3 Kg.) died suddenly after having received only 1·38 gm. of dry leaves in the course of ten days. This amount of leaves is less than the M.L.D. given in one dose. This could be regarded as a case of sensitization, unless we

accept that the animal possessed an idiosyncrasy for gifblaar; (b) *Rabbit R* (2·3 Kg.) is a case similar to Rabbit C. The former died from gifblaar poisoning after having received 1·15 gm. of dry leaves in the course of five days. This amount of plant is much less than the M.L.D. of dry leaves administered in one dose; *Rabbits K, L, N, P,* and *Q* died after having received in the course of a number of days amounts of dry leaves slightly higher than the M.L.D. given in one dose.

An experiment similar to the above was conducted with the dried underground stems of gifblaar collected by Dr. A. C. Leemann (of the Division of Plant Industry, Pretoria) at Rietondale, Pretoria.

Material was collected at intervals of one week from the 17.7.33 when the plant was in the dormant state, to 1.9.33 when it had produced a large number of leaves. In all, seven collections were made. Each consignment of underground stems were dried and the M.L.D. per Kg. rabbit determined. Leemann considered it likely that in the dormant state the underground stems of the plant contained precursors of the active principle of the plant and that these precursors when brought into the animal body may induce the development of a tolerance to the plant.

The first consignment of plant material was given to two rabbits, and for each further consignment two rabbits were added, so that for the seven collections fourteen rabbits were employed. In order to explain the manner of dosing the collections are numbered 1, 2, 3, 4, 5, 6 and 7, and the rabbits A to N. Rabbits A and B received consignment 1 the first week, consignment 2 the second week, and so forth. Rabbits C and D did not receive consignment 1, but all consignments from the second collection onwards. Rabbits E and F did not receive consignments 1 and 2, but all from the third collection onwards, and so forth. Rabbits M and N therefore only received consignment 7. All the rabbits were drenched every alternate day from the time they were placed in the experiment. The initial dose, which was equivalent to one-tenth M.L.D. per Kg. body-weight, was slightly increased at weekly intervals until about ½ M.L.D. was reached. Each rabbit was drenched for about six weeks and the tolerance test applied five days after discontinuation of the preliminary drenching experiment. In the tolerance test the animals received 2 M.L.D. of gifblaar leaves.

The results of this experiment were identical with those described in the former experiment, namely (*a*) there was no evidence of decreased susceptibility to gifblaar after preliminary dosing with small quantities of the underground stem; and (*b*) there was evidence of increased sensitivity to the plant and very slow inactivation, or (and), excretion of the active principle.

The interesting observation was made that there was a continuous decrease in the M.L.D. of the dried underground stems per Kg. rabbit from 5·3 gm. on 1.7.33 to 50 gm. on 17.9.33. This phenomenon appears to indicate that the active principle is concentrated in the underground portions of the plant while in the dormant state and that a high percentage of the active principle is passed into the leaves as soon as these appear. A definite opinion in regard to this phenomenon can, however, only be expressed when experiments were conducted upon the same plant.

Summary.

From the results of experiments conducted upon thirty-two rabbits it appears (*a*) that the continuous ingestion of leaves or underground stems of gifblaar does not induce the development of tolerance to this plant; and (*b*) that the active principle of gifblaar has cumulative effects, that is, it is inactivated in the body or excreted at a very slow rate. It is also possible that repeated small doses of the plant may cause progressive damage to organs of vital importance (heart) and that the sum total of these consecutive and progressive lesions is sufficient to cause death in spite of the fact that the active principle has been partly or completely excreted.

There also was a certain amount of evidence that some animals became sensitized to the effects of the plant, unless we accept that these animals possessed an idiosyncrasy to gifblaar.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 3, Number 1, July, 1934.

Recent Investigations into the Toxicity of Known and Unknown Poisonous Plants in the Union of South Africa.

By

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(Continued from *Onderstepoort Jour. of Vet. Sci. and Anim. Ind.*, Vol 1, 1933.)

ASCLEPIADACEAE.

Trichocaulon piliferum (L.f.) N.E. Br. (O.P.H. No. 4319; 14.7.33).

Common name.—Ghaap.

Origin.—Barrydale, Cape Province.

Uses.—It is used as a stomachic. The plant has a bitter taste.

State and Stage of Development.—Fresh; no flowers or fruits were present.

Rabbit.—Received 50 gm. of the fresh plant by stomach tube on 15.7.33.

Result.—16.7.33; another 50 gm. of fresh plant. The animal developed no symptoms of poisoning.

AMARYLLIDACEAE.

Buphane disticha (Linn. f.) Herb. (O.P.H. No. 6919; 13.10.33) = *Buphane toxicaria* Herb.

Common Names.—Gifbol, seeroogblom.

Origin.—Nongoma, Zululand.

State and Stage of Development.—Fresh; no flowers or fruits present.

Rabbit.—Received 10 gm. of fresh bulb and leaves by stomach tube.

Result.—Negative.

Rabbit.—Received 80 gm. of fresh bulb and leaves by stomach tube.

Result.—Fifteen minutes after having been drenched the animal exhibited pronounced dyspnoea and restlessness. The heart-action was extremely accelerated and weak. Death occurred 30 minutes after drenching.

Post Mortem Appearences.—Pronounced hyperaemia and slight oedema of the lungs; heart in systole.

COMPOSITAE.

Epaltes alata Steetz (O.P.H. No. 3891; 19.6.33; N.H.P. No. 15300).
Distribution.—Pietersburg, Potchefstroom, Bloemhof, Schweizer Reineke, Heidelberg, Waterberg, Transvaal; Kroonstad, Heilbron, Orange Free State; Vryburg, Warrenton, Cape Province; Bechuanaland; Southern Rhodesia; Portuguese East Africa.

Active Principle.

Unknown.

Toxicity of the Plant.

No records of the toxicity of the plant could be found in the literature consulted. The author conducted the following experiments at Onderstepoort: A full-grown sheep, which had received 500 gm. of the fresh plant (collected in the Bloemhof District, Transvaal, in the flowering stage) per os. on each of two consecutive days developed no clinical symptoms (Steyn, 1929). The following experiments were conducted with the plant in the late flowering and seeding stage collected in the Heilbron District, Orange Free State: (*a*) 400 gm. of the fresh plant followed by 200 gm. on the succeeding day induced transient symptoms of poisoning in a sheep; (*b*) a full-grown sheep, which had received 100 gm. of the dry plant on each of four consecutive days followed by 200 gm. on each of five consecutive days, died on the ninth day of the experiment; (*c*) a full-grown sheep, which had received 1,500 gm. of the dry plant in the course of thirty-six hours, died twelve hours after administration of the last dose; (*d*) 30 gm. of the dry plant administered daily for eleven days had no ill effects on a full-grown sheep; (*e*) a rabbit (2·2 Kg.), which had recived 10 gm. of the dry plant daily for eleven days, and (*f*) a rabbit (2·1 Kg.), which had received 10 gm. daily for six days followed by 40 gm. daily for four days, developed no symptoms of poisoning.

Symptoms of Poisoning.

Sheep.—The animal, which had received 600 gm. of the fresh plant (see " Toxicity of the Plant ") on two days, developed the following symptoms on the fourth day after having received the second dose: paralysis of the front quarters, imperceptible pulse, dyspnoea (accelerated and abdominal breathing), cyanosis of visible mucous membranes and conjunctivae, loss of appetite, and apathy. On the following day the animal managed to rise with difficulty. There was pronounced ataxy of the fore-legs. Fever, apathy, loss of appetite, weak and accelerated pulse, and dyspnoea were present. On the fourteenth day of the experiment the animal appeared normal with the exception of slight weakness in the front quarters. Loss in condition of the animal had occurred in the meantime. Larger amounts of the plant caused death in sheep forty-eight hours after the first dose. The sheep, which had received 100 gm. of the dry plant daily for four days followed by 200 gm. daily for five days developed clinical icterus in addition to the above symptoms. Temperatures as high as 107° F. were recorded.

Post Mortem Appearances.

General cyanosis; slight hydroperitoneum and hydropericardium; pronounced oedema and hyperaemia of the lungs; subepicardial and subendorcardial haemorrhages; degenerative changes in the myocard; pronounced degenerative changes in the liver (boiled appearance). In more protracted cases there was slight general icterus, extensive fatty degeneration with slight pigmentation of the liver, and haemorrhages in and ulceration of the abomasal mucosa.

Senecio bupleuroides D C. (O.P.H. No. 9698; 7.12.32; N.H.P. No. 14384).

Origin.—Potgietersrust, Transvaal.

State and Stage of Development.—Fresh and in the flowering stage.

Sheep 35330.—Received 400 gm. of fresh leaves, stems and flowers on each of two consecutive days.

Result.—Negative.

DICHAPETALACEAE.

Dichapetalum cymosum (Hook.), Engl.

Common Names.—Gifblaar, blaargif, blinkblaar.

Origin.—Rietondale, Pretoria.

As stock-owners report favourable results in connection with the treatment of gifblaar poisoning with vinegar and kaffir-beer, it seemed desirable to ascertain whether an acid environment had any detrimental effect on the active principle of this plant. An experiment was conducted on the following lines: (*a*) The M.L.D. of an aqueous extract of the dried root per Kg. body-weight of rabbit was determined; (b) an aqueous extract of the dried root was rendered acid by the addition of acetic acid and left standing for five hours before administration to rabbits; and (*c*) an acid aqueous extract of the dried root was prepared by extracting for twenty-four hours with distilled water acidified with acetic acid.

The aqueous extract prepared by immersing the dried root in distilled water was neutral.

The M.L.D. of each of the above extracts per Kg. body-weight of rabbit was 15 cc. (equivalent to 2·5 gm. dry root). An acid environment therefore appears to have no detrimental effect on the active principle of gifblaar.

GENTIANACEAE.

Enicostemma littorale Blume (O.P.H. No. 15,111; 3.3.33).

Origin.—Modderfontein, P.O. Zebediela, Transvaal.

State and Stage of Development.—Fresh and in early flowering stage.

Rabbit.—Received 30 gm. of fresh flowers, leaves and stems by stomach tube.

Result.—Negative.

Homeria pura N.E. Br. (O.P.H. No. 5281; 27.9.32) (N.H.P. No. 10572).

Common Names.—Yellow tulip, geel tulp.

Origin.—Left bank of Vaal River, Vredefort District, Orange Free State.

Active Principle.

Unknown.

Toxicity of the Plant.

No reference to the toxicity of the plant could be found in the literature consulted. The author conducted experiments with the fresh leaves of the plant in the flowering stage. A full-grown rabbit, which had received 15 gm. of the fresh leaves, developed paralysis of the fore-quarters, accelerated and weak heart-action, and pronounced dyspnoea, and died one and a half hours after administration of the plant. A second rabbit died one hour after having received 30 gm. of the fresh leaves.

Autopsy revealed general cyanosis, pronounced dilatation of both ventricles of the heart, and congestion of the liver and lungs.

LILIACEAE.

Albuca sp. (O.P.H. Spec. 6726; 3.10.33) (N.H.P. No. 15726).

Origin.—Kliprivierśval, P.O. Meyerton, Johannesburg.

State and Stage of Development.—Fresh and in flowering stage.

Rabbit.—Received 40 gm. of fresh bulbs, leaves and flowers by stomach tube.

Result.—Twenty hours after having been drenched the animal was listless, took no food and suffered from diarrhoea. Death occurred about thirty-six hours after drenching.

Post Mortem Appearances.—Hyperaemia of the lungs, acute catarrhal gastritis with pronounced hyperaemic patches on mucosa; patchy hyperaemia of mucosa of small intestine.

Ornithogalum Saundersiae Baker (O.P.H. No. 6728; 3.10.33).

Origin.—Stainton, Ixopo, Natal.

State and Stage of Development.—Fresh and in pre-flowering stage.

Rabbit.—40 gm. of fresh leaves and bulbs by stomach tube.

Result.—Sixteen hours after having been drenched the animal appeared listless, took no food and was purging. Death occurred about thirty-six hours after drenching.

Post Mortem Appearances.—Acute catarrhal gastritis with pronounced hyperaemic patches; large amount of fluid in intestine.

PHYTOLACCACEAE.

Giesekia pharnaceoides L. (O.P.H. No. 2710; 13.5.33).

Origin.—Pietersburg, Transvaal.

State and Stage of Development.—Dry and in seeding stage.

Rabbit.—Received 20 gms. of dry plant on each of three consecutive days with negative results.

Oxygonum sinuatum (Hochst.) Dam. (O.P.H. No. 2712; 13.5.33).

Origin.—Pietersburg.

State and Stage of Development.—Dry and in seeding stage.

Rabbit.—Received 20 gm. of dry plant on each of three consecutive days with negative results.

SOLANACEAE.

Cestrum laevigatum Schlecht. (O.P.H. 4485; 27.7.33).

Common Name.—Inkberry.

Origin.—East London.

State and Stage of Development.—Fresh and in flowering and early fruiting stage.

Rabbit.—Received 30 gm. of fresh young shoots, flowers and immature fruit with negative results.

Solanum supinum Dunal (O.P.H. No. 16248; 3.4.33).

Common Name.—Bitterappel; bitter apple.

Origin.—P.O. Middelfontein, Waterberg.

State and Stage of Development.—Dry plant with immature and mature fruit.

Immature Fruit.—Two rabbits received 10 gm. and 120 gm. (in two doses) of fresh immature fruit respectively with negative results.

Mature Fruit.—Two rabbits received 10 gm. and 120 gm. (in two doses) of fresh mature fruit respectively with negative results.

ZYGOPHYLLACEAE.

Zygophyllum foetidum Schrag. (O.P.H. No. 5054; 8.8.33).

Common Name.—Skuimbos.

Origin.—Kruitfontein, Somerset East.

State and Stage of Development.—Wilted and in early flowering stage.

Rabbit.—Received 15 gm. of wilted leaves on each of two consecutive days with negative results.

Rabbit.—Received 60 gm. of wilted leaves in two doses on one day, and 30 gm. on the following day.

Result.—On the fourth day after the last dose the animal was found paralysed and showing continuous clonic spasms of the muscles of the nose, lips and different parts of the body. The legs were moved continuously. The animal appeared unconscious and died after having been in this state for twenty hours .

Post Mortem Appearance.—Heart in systole; hyperaemia of the lungs; urinary bladder markedly distended with urine.

Sheep 37169.—Received 500 gm. of wilted leaves in one dose with negative results.

The experiment had to be discontinued owing to lack of material. The sender of the plant wrote (*a*) that the plant is more poisonous in winter than in summer; (*b*) that stock must be very hungry for the plant to exert its effects; (*c*) that the plant is poisonous only when it grows in the shade; and (*d*) that it is most poisonous in the early morning.

SUMMARY.

The toxicity of fourteen plants were investigated. The symptoms and post mortem appearances caused by three plants, which have previously not been proved poisonous, are described. These three plants are (*a*) *Epaltes alata* Steetz. (*b*) *Homeria pura* N.E. Br. and (*c*) *Albuca* sp. (O.P.H. No. 6726, 3.10.33; N.H.P. No. 15726). According to the results of the experiments conducted no definite opinion as to the toxicity of *Zygophyllum foetidum* Schrag. can be expressed. An acid environment has no effect on the active principle of gifblaar in the course of twenty-four hours.

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Section IV.

Physiology.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 3, Number 1, July, 1934.

Studies on Sex Physiology.

XI. The Relationship between a Corpus Luteum Verum and the corresponding Pregnant Horn.

By H. H. CURSON, Dr.Med.Vet., F.R.C.V.S., Veterinary Research Officer, Onderstepoort.

In the previous study the situation of the developing foetus in the Merino sheep was investigated. Using the same material, observations were made concerning the pregnant horn and the relative corpus luteum verum. These are set down hereunder.

It will be remembered that of 41 pregnancies, 5 were double and 36 were single. It now remains to make an analysis of each of these groups.

Double Pregnancies.

Of the 5 sheep, the *usual* state of affairs was seen in three cases, namely, the foetus was situated in the horn corresponding with the ovary from which the ovum in question arose. It is, of course, possible that each ovum migrated into the opposite horn for development, but there is no method of checking this. It is, therefore, assumed that the foetus of one side corresponds to the corpus luteum verum of the same side.

In the two remaining cases (Nos. 31 and 35) *migration* had definitely occurred, for both ova in each case had been liberated from the left ovary and yet a foetus existed in *each* horn. The relative diagram represents migration where both corpora lutea vera are to be found in the right ovary. See Figure.

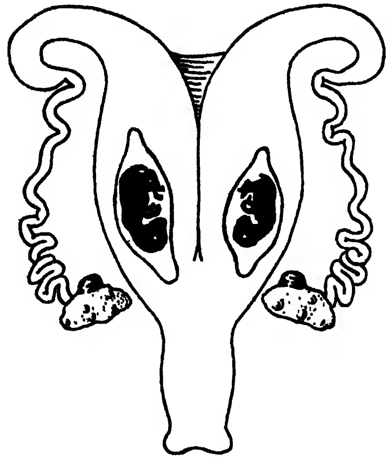
Single Pregnancies.

Of the 36 sheep, the *usual* position was noted in all instances, but two (Nos. 26 and 34). In 15 cases it was the left ovary that was responsible for the initial ovulation, and in the remaining cases (19), the corpus luteum verum was to be found in the right ovary.

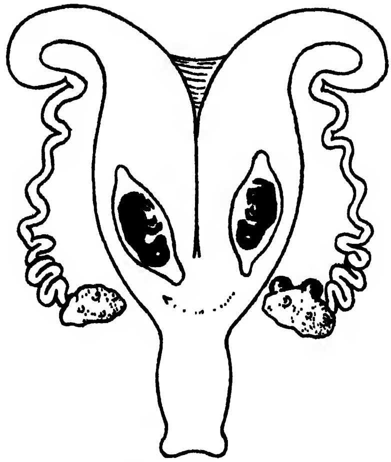
In sheep Nos. 26 and 34 the left ovary contained the corpus luteum verum but the corresponding pregnancy had taken place in the right uterine horn. The relative diagram illustrates the position as occurring in these two sheep.*

* My colleague, Quinlan, J., in a triple pregnancy in a Swiss goat, observed (10.8.33) 3 corpora lutea in the right ovary. The kids were arranged, one in right horn (anterior presentation), one in left horn (posterior presentation), and the third transversely in pars indivisa with the ventral surface caudal.

DOUBLE.

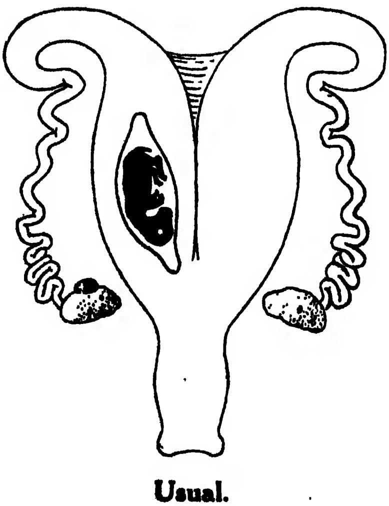


Usual.

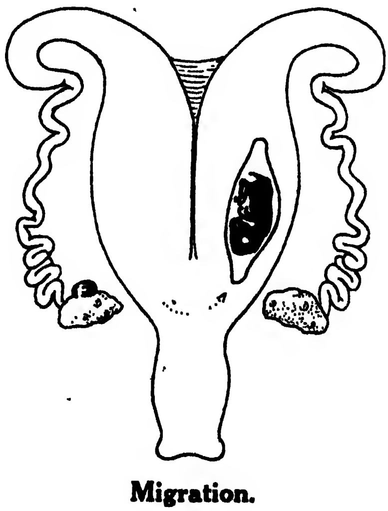


Migration.

SINGLE.



Usual.



Migration.

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Section V.

Studies in Photosensitisation.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 3, Number 1, July, 1934.

Studies on the Photosensitisation of Animals in South Africa.

VII The Nature of the Photosensitising Agent in Geeldikkop.

By

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INTRODUCTION.

In the first article of this series it was indicated that outbreaks of "geeldikkop" amongst small stock in the Karroo areas could definitely be ascribed to excessive feeding on *Tribulus* during certain periods of the year. Beyond this, very little is known about the factors rendering the plant toxic or of the toxic principles concerned. This is primarily due to the insidious nature of the disease and the difficulty of carrying out experiments under natural conditions. Thus a pasture which is generally considered excellent for sheep, suddenly becomes extremely toxic. Furthermore, the problem is complicated by the fact that outbreaks of a disease, which appears in all respects to be identical with geeldikkop, may at times be encountered on grass pastures and even in lucerne paddocks where *Tribulus* can be excluded. When, however, any of these suspected plants, including *Tribulus*, are fed to sheep under laboratory and even under field conditions, the results usually obtained are either negative or insignificant. Where watery extracts of *Tribulus* are dosed, as shown in the second article, death may result from methaemoglobinuria. This was found to be due to the large amount of nitrite present in such extracts. Clinically, however, geeldikkop appears with a sudden onset of oedema of the exposed and unpigmented parts of the head and accompanied by definite signs of photosensitisation. This is soon followed by a progressive generalised icterus first evident on the visible mucous membranes, and subsequently on the skin. The urine too is deep yellow. In chronic cases the affected skin of the face and ears undergoes necrosis and subsequent sloughing. The blood serum by this time is deep yellow and shows a strongly positive direct van den Bergh reaction. At post-mortem, the most striking finding is the intense icterus, together with enlargement of the gall bladder, although the common bile duct is always found patent. The liver especially is deeply bile-stained.

The problem therefore resolved itself into an investigation of the nature of the photosensitising and icterogenic factors and the genesis of the symptoms and lesions seen in the disease. Once this was achieved it was hoped that rational means of prophylaxis would present themselves.

On account of the disappointing results obtained with plant material supposed to be toxic, one was forced to approach the problem by various indirect means. Reports of this series of investigations were published in the Onderstepoort Journal of Veterinary Science and Animal Industry, Vol. I, No. 2.

In the 6th article, the photosensitisation following ligation of the bile ducts in sheep was described, the cause of which, however, was unknown at the time. Since then, further work on this phenomenon of photosensitisation has been carried out on the bile, blood and faeces of operated sheep as well as on the same materials collected from naturally occurring cases of geeldikkop. In this article it is intended to report upon the findings which led to the identification of the photosensitising principle.

Photosensitisation, or acute sensitivity of the exposed parts of the body to the light of the sun, is a phenomenon well known to occur after the injection of many fluorescent dyestuffs, including the porphyrins, and also following the ingestion by animals of certain green plants. Of the latter, various species of *Hypericum* (St. John's wort) and of *Fagopyrum* (buckwheat) are best known. In the case of the former, it has been proved experimentally that there is present in the plant a pigment ("hypericin") which is capable of causing direct photosensitivity when injected into the blood stream.

Tribulus plants, including specimens taken from a farm where geeldikkop was active, were examined for the presence of fluorescent colouring matters of the hypericin type, but no trace of such could be found. The photosensitising pigment had consequently to be looked for in the animal's body, not in the external plant material.

THE PHOTOSENSITISING FACTOR IN EXPERIMENTAL BILIARY OBSTRUCTION CASES.

In these investigations sheep were used as experimental animals and the operations carried out as described elsewhere. The animals' diet consisted of fresh green lucerne supplemented by a little dry hay and crushed maize. Blood was withdrawn from animals exhibiting sensitivity and also from control normal sheep in the same camp and an examination made for porphyrin-like substances. In other cases, sensitive and control animals were slaughtered and the chief organs worked up for porphyrin. The methods employed were largely those of Fischer and his collaborators in their post-mortem investigation of the human porphyrinuric Petry, or procedures based upon these. (Fischer, Hilmer, Lindner and Pützer, 1925; Fischer and Zerweck, 1924.)

Typical experiments are the following:—

Comparison of blood of normal and sensitive sheep from the same camp. Three sensitive and three controls used yielding 500 c.c. of blood from each group. Serum and corpuscles worked up separately.

Corpuscles: Normals. Extract in 1 c.c. of ether shows ± fluorescence, no spectrum.

Sensitive. Extract in 2 c.c. of ether showed red fluorescence in ultra-violet light: absorption spectrum * was—

in ether 635; 600–575; 565–555; 525–520 Order III, II, IV, I;
I II III IV

in 25% HCl 600–595; 570 Order II, I.
I II

* Absorption spectra measured by a Zeiss direct-vision pocket spectroscope. Liquid layer 2 cm.

Serum: Normals. Extract in 0·5 c.c. ether: no fluorescence, no spectrum.

Sensitive. Ether extract shows deep red fluorescence and following absorption spectrum—

630; 595; (583); 578; 557; 527–525
I II III IV

Order III, II, IV, I.

On another occasion, 19 c.c. of serum from the same three sensitive sheep was worked up with the following results:—

Extract in 2 c.c. of ether was brownish pink in colour and showed an intense red fluorescence. Spectrum:

635; (620); 600–590; 565–555; 520 Order III, II, IV, I.
I II III IV

The pigment was esterified in methyl alcoholic hydrochloric acid solution and crystallised from chloroform-methyl alcohol yielding a small quantity of microscopic prisms.

In chloroform solution, these exhibited the following spectrum:

632; 595; 577; 560 Order III, II, I.
I II III

The characteristics of this pigment, present only in the blood of sheep which are actually photosensitive at the time of bleeding or slaughter, indicate that it belongs to the class of porphyrins.

It was also demonstrated that in sensitive animals the photoactive pigment is confined almost entirely to the serum, well washed corpuscles yielding only a trace of a porphyrin in all probability identical with protoporphyrin (compare Hymans van den Bergh, Grotepass and Revers, 1932).

The result of the examination of organs taken from sensitive and non-sensitive animals was unfruitful. In no case was an unusual pigment detected.

Some comparative experiments were made with sheep poisoned by the administration of lead acetate. In these cases examination of the blood revealed the presence of considerable quantities of a porphyrin having the properties, absorption spectra, etc., of protoporphyrin. In no case did the animals exhibit photosensitivity.

Attention was next directed to an examination of the bile. Bile fistulae were introduced into a number of sheep and the secretion collected in bottles strapped to the animal's bodies, a little toluol being added to prevent putrefaction. It was found that even in normal bile, taken from the gall bladder at the time of operation, a small quantity of the same porphyrin-like material was present as had been detected in the blood serum of photosensitive sheep. After the operation in which the fistula was inserted, however, a pronounced rise in the concentration of this pigment was observed in nearly every case. The final concentration, reached in about 4 to 12 days, was anything from 3 to 20 times the normal pre-operative concentration of pigment.

Quantitative comparison was made in the following manner: A representative sample of normal bile was obtained by combining the contents of the gall bladders of healthy laboratory sheep passing through the post-mortem room. In all 450 c.c. was obtained and the porphyrin extracted from this by the usual method. From the hydrochloric acid solution, the pigment was passed back to ether and the volume of the ethereal solution adjusted to 450 c.c. A solution was thus obtained having a pale pinkish-brown colour and exhibiting a well-marked fluorescence in ultra-violet light. When examined in a 2 cm. layer, the absorption band at 560 $m\mu$ was just plainly visible but no trace was seen of the remainder of the absorption spectrum owing to the relative weakness of these bands. Such a solution (found afterwards to contain 12 mg. of the pure pigment per litre) was adopted as the comparison standard of normal bile and other bile samples evaluated by diluting the ethereal solutions of their contained porphyrin until the intensity of the 560 $m\mu$ absorption band matched that of the standard solution. Such a comparison is admittedly rather rough, but in view of the wide fluctuations encountered in porphyrin concentration, was deemed to be of sufficient accuracy. If, for example, the porphyrin from 20 c.c. of bile had to be diluted in ether, to 40 c.c. to match the standard, this concentration was spoken of as "2 bile units", and so on.

The following experiments are selected as illustrating, in a typical manner, the rise in bile porphyrin following the fistula operation. It will be noted that the significant increase occurred within just that space of time that was found requisite, previously, for bile-ligature animals to become sensitive.

Sheep 32979.

Gall bladder sample		Vol. 11 c.c.	contained	1·1	units of	porphyrin.
15·5 hrs. after operation		105 c.c.	,,	1·1	,,	,,
39·5	,, ,,	118 c.c.	,,	7·2	,,	,,
63·5	,, ,,	177 c.c.	,,	9·0	,,	,,
87·5	,, ,,	224 c.c.	,,	4·3	,,	,,
		Died.				

Sheep 28632.

Gall bladder sample	Vol. 5·5 c.c.	contained	1·6	units	of porphyrin.
18 hours after operation	150 c.c.	,,	1·4	,,	,,
84 ,, ,,	170 c.c.	,,	11·0	,,	,,
108 ,, ,,	180 c.c.	,,	11·0	,,	,,
	etc.				

Sheep 35329.

Gall bladder sample contained ...	1·4	units	of porphyrin.
6 hours after operation contained	1·5	,,	,,
24 ,, ,, ,,	3·0	,,	,,
2 days ,, ,, ,,	6·6	,,	,,
3 ,, ,, ,,	17·3	,,	,,
4 ,, ,, ,,	33·6	,,	,,
5 ,, ,, ,,	56·0	,,	,,
6 ,, ,, ,,	30·5	,,	,,
7 ,, ,, ,,	15·5	,,	,,
8 ,, ,, ,,	60·0	,,	,,
9 ,, ,, ,,	12·5	,,	,,
10 ,, ,, ,,	12·5	,,	,,
11 ,, ,, ,,	12·5	,,	,,

That some external condition such as diet was the factor responsible for the increase in porphyrin concentration after operation appeared most probable, and was at a later stage abundantly proved to be the case. It was our practice to offer the animals a liberal supply of green stuff immediately following the operation since on the ordinary ration they frequently lost appetite. Some incidental observations upon the bile of other animal species may be briefly recorded here. The findings are all in harmony with the thesis that the porphyrin originates from the green feed.

Horse bile. Sample taken post-mortem volume 5 c.c.; golden-brown in colour, mixed with a little blood. Extract in 2 c.c. of ether

showed 598; 560; 525 Order II, I, III
I II III

in 25% HCl 610; 565.

Porphyrin obtained in crystalline condition from chloroform.

Ox bile (No. 4225) sample taken post-mortem volume 120 c.c.; contained 3 units of porphyrin. Spectrum in ether

598–580; 560; 525 Order II, I, III.
I II III

Milk-fed calf. Sample taken from gall bladder at operation (ligature of bile duct). Porphyrin nil. Animal did not become sensitive.

Dog's bile. White bull terrier. Sample taken at operation (ligature of bile duct); spectrum in ether (greenish-blue).

660–630; 595–580; 535; (500). Animal did not become sensitive.

Cat's bile.—Sample taken post-mortem, combined from 4 young cats, volume 5 c.c. Porphyrin nil.

The characteristics of the porphyrin present in normal and fistula sheeps' bile were as follows:—

Normal sheeps' bile. Spectrum in ether 635; 593; 578; 560; 525
I II III IV

Order III, II, IV, I.

In 25% HCl 605; 570 Order II, I.
I II

Fistula bile. Spectrum in ether 630; 595; 578; 558; 525.
I II III IV

Order III, II, IV, I.

In 25% HCl 620–600; 580–555 (525) Order II, I.
610 570
I II

The pigment passed completely into chloroform from hydrochloric acid solution on repeated shaking with the solvent. The chloroform solution was a deep purplish-crimson in colour and left the pigment in a crystalline state on evaporating spontaneously at room temperature. Washing of the residue with ether removed a small amount of accompanying pigment showing a pronounced absorption band in the region of 650 $m\mu$. The porphyring itself was insoluble in ether, sodium carbonate, water or alcohol, very sparingly soluble in chloroform but readily soluble in glacial acetic acid or in pyridine.

That this pigment was actually responsible for the photosensitisation noticed in the experimental animals was proved by tying off the fistula tube and so causing an obstruction icterus as exemplified in the following experiment:—

Sheep 35326 was operated upon and a fistula tube inserted into the ductus choledochus. The secretion of bile was collected in a bottle and analysed daily. After some days the porphyrin concentration was 9 units and remained at this level without sign of alteration. The spectrum in ether was 630; 595; 578; 558; 525. The fistula tube was then closed externally by ligature. Within 24 hours the animal had become markedly photosensitive, the blood plasma was yellow in colour and gave a direct van den Bergh reaction, whilst signs of clinical icterus were also visible. 7 c.c. of serum was worked up for porphyrin and sufficient pigment obtained to show the following spectrum in about 0·5 c.c. ether:—

596; 578; 560; 525–520.

It fluoresced strongly in ultra-violet light.

THE PHOTOSENSITISING PIGMENT IN GEELDIKKOP.

At about this stage in the work, the opportunity arose of investigating a fairly severe outbreak of geeldikkop on the farm Dassiespoort, in the Fauresmith area of the Karroo. Although the disease had nearly subsided by the time the affected area was reached, we were able to select several cases typical of the chronic, advanced

stage with sloughing lips and ears, also some animals still in the earlier stages. Specimens were slaughtered and samples brought back to the laboratory for chemical examination. Examination of the *Tribulus* growing in the affected paddocks, showed that the plants were small and stunted, moderately parasitised by a weevil-like grub but otherwise in no way peculiar. Chemical tests for alkaloids and nitrites were negative. It was not possible to carry out extensive feeding trials.

The chemical post-mortem findings on the animals may be summarised as follows (examination of the organs were negative; description therefore omitted):—

Blood serum: In every case examined, deep yellow. Positive direct van den Bergh reaction.

Case 1 (of approximately 48 hours standing). Serum from 660 c.c. of blood worked up for porphyrin. Extract in ether had a brownish colour and exhibited well marked fluorescence in ultra-violet light. Spectrum $\underset{\text{I}}{630};\ \underbrace{595;\ 577}_{\text{II}};\ \underset{\text{III}}{560};\ \underset{\text{IV}}{525}$ Order III, II, IV, I.

Case 2 (chronic). Serum from 660 c.c. of blood worked up for porphyrin. Ether extract pinkish-brown; strong fluorescence.

Spectrum $\underset{\text{I}}{633};\ \underbrace{595;\ 578}_{\text{II}};\ \underset{\text{III}}{560};\ \underset{\text{IV}}{523}$ Order III, II, IV, I.

The combined pigment from cases 1 and 2 was esterified in methyl alcohol-hydrochloric acid and passed into chloroform. Upon evaporation, the chloroform solution deposited a small quantity of microscopic prisms but the amount was too small to permit of analysis.

Corpuscles: The red-cell volume in all cases was found to be normal. There was no indication of any extensive haemolysis having taken place.

Oedema fluid: 30 c.c. of yellow fluid obtained from the intra-mandibular space was examined for porphyrin but the result was negative.

Bile: The chemical examination of the bile afforded very striking results. From the gall bladder of case 2 was obtained 25 c.c. of dark, brownish-green, rather viscid bile. This was worked up for porphyrin in the usual way. The final ether solution (100 c.c.) was of an intense rose-red colour and exhibited the following spectrum:—

$$\underset{\text{I}}{635};\ \underbrace{593;\ 578}_{\text{II}};\ \underset{\text{III}}{565\text{–}555};\ \underset{\text{IV}}{530\text{–}515}\ \text{Order III, II, IV, I.}$$

It was found that a concentration of 9 per cent. of hydrochloric acid was necessary to remove most of the pigment from the ether (i.e. its acid number was in the region of 9).

In 25 per cent. HCl the spectrum was:—

610–600; 595; 580–555 Order III, I, II.
I II III

On evaporating the acetic acid-ether solution of the pigment at room temperature, a crystalline deposit of fine prisms was obtained (see Fig. I). These, together with a similar crop from bile of case 1, were converted into the methyl ester, freed from fatty material by washing with petroleum ether, and taken up in chloroform.

Spectrum of ester in chloroform 630; 595; 560; 530–525 Order III, II, IV, I.
I II III IV

Fig. 1.—Porphyrin from dikkop bile (Dassiespoort) crystallised from acetic acid ether. Magn. 175×.

From the gall bladder of case 1 was obtained 20 c.c. of bile similar in appearance to that of case 2. It was very rich in porphyrin, the ether solution exhibiting the following spectrum:

635; 598; 580; 560; 530–520; (492) Order III, II, IV, I.

I II III IV

The crude pigment could be extracted from 25% HCl by shaking with chloroform and was deposited from this solvent in clusters of fine needle-like crystals very similar in appearance to those similarly obtained from fistula bile crude porphyrin (see Fig. 4).

Urine: The urine from all three cases appeared to be free from porphyrin, although containing much bile pigment.

The above results with the bile from these two geeldikkop cases were amply confirmed when an examination was made of specimens secured during a subsequent outbreak at Middelburg, C.P. The quantities of porphyrin were evaluated in terms of the arbitrary "normal" standard and were found to range from 4 to 13 units, as follows:—

Case No.	*Volume of Bile, c.c.*	*Porphyrin Concentration in "Units".*
1	66	11·0
2	56	8·3
3	63	8·4
4	127	8·0
5	52	8·0
6	34	10·4
7	20	8·5
8	100 ("yellow bile")	3·8
9	97	10·0
10	33	6·0
11	59	12·5
12	60	10·0
13	5 (no clinical icterus: "yellow bile").	4·2

It was concluded from these experiments that the pigment causing photosensitivity in geeldikkop is identical with that found to be responsible for this symptom in the experimental, biliary-obstruction cases produceable at the laboratory. In addition, in both instances, the porphyrin content of the bile was found to be high, considerably higher than in sheep subsisting upon the usual diet supplied to the available pen animals.

ISOLATION OF THE PHOTOSENSITISING PIGMENT.

Clearly, the most readily available source of the pigment for isolation purposes was fistula bile, consequently the isolation and chemical identification of this pigment was approached as the next step in the logical elucidation of the geeldikkop problem. Quantities of bile amounting to 12·5 litres in all were obtained from fistula sheep. The porphyrin concentration was such that this quantity was equivalent to 67 litres of normal bile. It was evaporated to dryness by fanning thin layers exposed in large shallow trays. The residue was extracted repeatedly with pyridine until this solvent was no longer coloured pink, some glacial acetic acid was then added to the pyridine, followed by a considerable quantity of ether and sufficient water to cause the separation of two phases. Great difficulty

was experienced in washing out the pyridine and water-soluble materials from the ether owing to the tendency to form emulsions; however, by cautious shaking with consecutive quantities of water this was achieved. The entire pigment was then transferred to 10% hydrochloric acid and ether and lipoidal impurities removed from this solution by aeration. After again transferring to ether, the crude porphyrin was fractionated by shaking with different concentrations of hydrochloric acid. The 8-10% fraction was purified by frequent transferences, finally taken into chloroform and this solution evaporated. The crystalline residue was taken up in a little warm pyridine and, after filtration, about 4 volumes of boiling methyl alcohol added and a drop of glacial acetic acid. Upon cool-

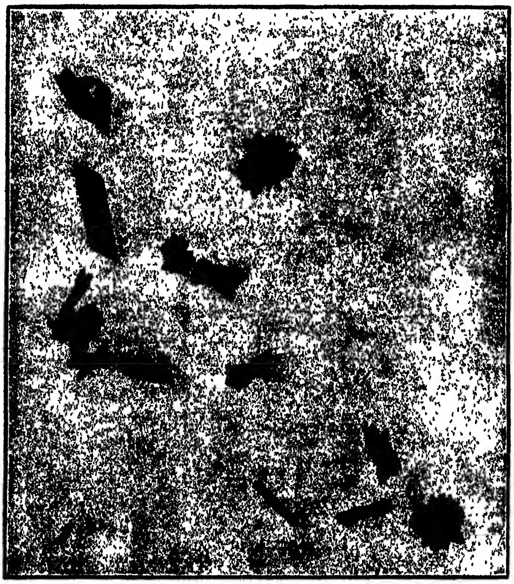

Fig. 2.—Bile porphyrin crystallised from pyridine-methyl alcohol. ×270.

ing, a fine crop of crystals was deposited. These were centrifuged off, washed well with ether and alcohol and finally recrystallised from hot pyridine-methyl alcohol. The pigment was thus obtained (in a yield of 0·3 gm.) in fine, large obliquely-ended prisms having a metallic lustre when seen in quantity (see Fig. 2). Other crystalline forms were obtained by crystallising from neutral ether (an acetic-ether solution washed repeatedly with water until freed from acid), which yielded tufts of very fine needles or needle-like prisms (see Fig. 3), and from acetic acid-ether solution on evaporation when the pigment was deposited in the form of slender, obliquely-ended prisms (see Fig. 4).

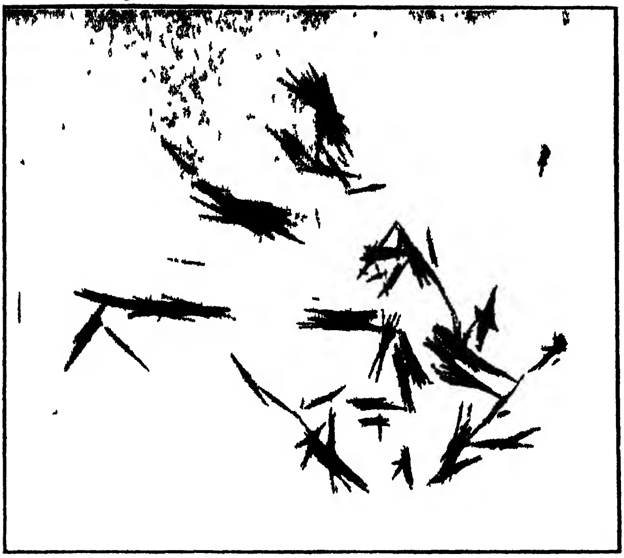

Fig. 3.—Porphyrin from fistula bile crystallised from neutral ether. ×170.

Fig. 4.—Porphyrin from fistula bile crystallised from acetic acid ether. ×270.

These three crystalline forms were shown to be interconvertable and to yield similar analytical figures.

The acid number of the pigment was shown to be 9. The absorption spectra were:—

In ether 635; 595; 578; 560; 523 Order III, II, IV, I.
I II III IV

in 25% HCl 620; 607; 568 Order II, I.
I II

Its methyl ester was prepared by suspending some of the crystalline pigment in absolute methyl alcohol and saturating the ice-cooled mixture with gaseous hydrochloric acid. Chloroform was then added and a considerable quantity of ice-water. After shaking, the chloroform layer was removed, washed repeatedly with dilute alkali and water to remove the excess of acid and finally concentrated to dryness. The residue was taken up in a little anhydrous chloroform and filtered; five volumes of hot methyl alcohol were then added and the ester allowed to crystallise. It was obtained in fine prisms M.P. 259° (see Fig. 5). Combustion micro-analyses were performed upon the preparations of free pigment and of the methyl ester. From the collected results the pigment was identified with phylloerythrin. This was confirmed by preparing a sample of the methyl ester of the latter and demonstrating that the melting point was not lowered by admixture of the methyl ester of the bile pigment. The analytical figures for carbon content are somewhat low, but it is well known that phylloerythrin is very difficult to combust quantitatively and always tends to give low figures (in this connection compare Noack and Kiessling, 1930). The pertinent data are recorded below:—

		C	H	N	CH_3O
Micro-analysis:	Bile pigment from pyridine	72·26	7·02	9·69	
	Bile pigment from ether	72·16	6·52	10·07	
	Phylloerythrin $C_{33}H_{34}N_4O_3$ requires	74·15	6·37	10·49	
	Bile pigment methyl ester (M.P. 259°) ...	73·08	6·59	11·08	6·06
	$C_{34}H_{36}N_4O_3$ (M.P. 262°) requires	74·45	6·57	10·22	5·65

The ethyl ester of the bile pigment melted at 245°, whereas that of phylloerythrin (from faeces) is given as 248°.

The absorption spectra of phylloerythrin are as follows:—

In ether (Hellström 1931) 635·2; 595; 589·8; 558; 526·6–512·3
I II III IV
Order III, II, IV, I.

In 25% HCl 619; 607; 577 Order II, I.
I II

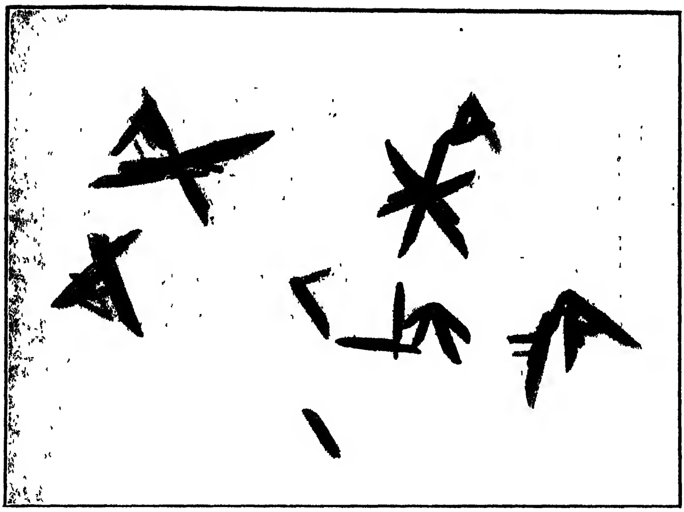

Fig. 5.—Methyl ester of porhyrin from fistula bile, crystallised from chloroform-methyl alcohol. M.P. 259°. 180×.

The relative intensity of the band at 558 $m\mu$ is well brought out in the figure which Hellström gives comparing the spectra of phylloerythrin, mesoporphyrin and phaeoporphyrin a_5, and from our photographs taken with a Zeiss grating instrument and achromatic plates. Figs. 6 and 7.

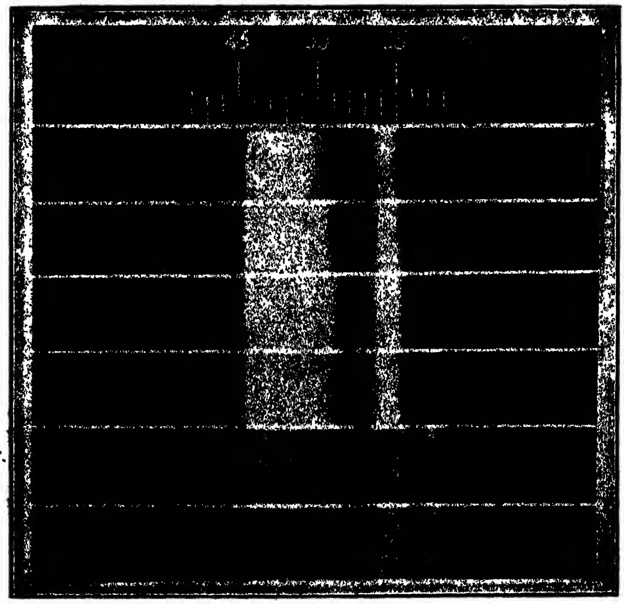

Fig. 6.—Absorption spectrum of phylloerythrin in ether solution

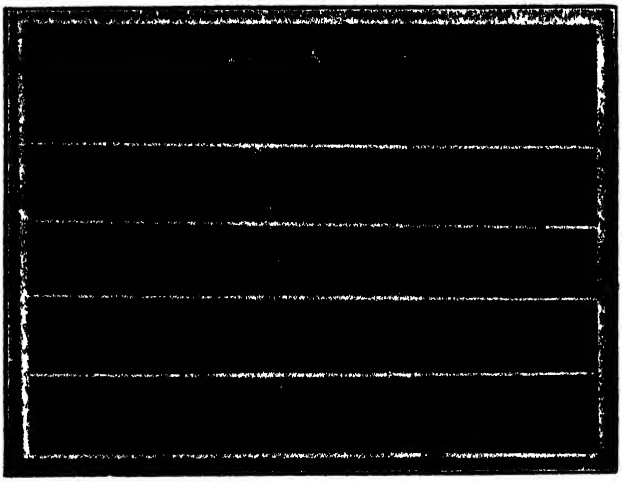

Fig. 7.—Absorption spectrum of phylloerythrin in 25 per cent. HCl.

A 0·1 gm. sample of the crystalline material from the dried bile was injected, dissolved in a little pyridine, intravenously into a sheep. Well marked photosensitisation resulted, the animal flinching, crouching, and eventually lying down in a most contorted position (see Figs. 8 and 9). Next day the head and ears were markedly swollen and a pouch of oedematous fluid distended the loose skin in the region of the intermandibular space. No sign of icterus was observed (Fig. 10).

Fig. 8.—Sheep showing photosensitisation after injection of bile porphyrin.

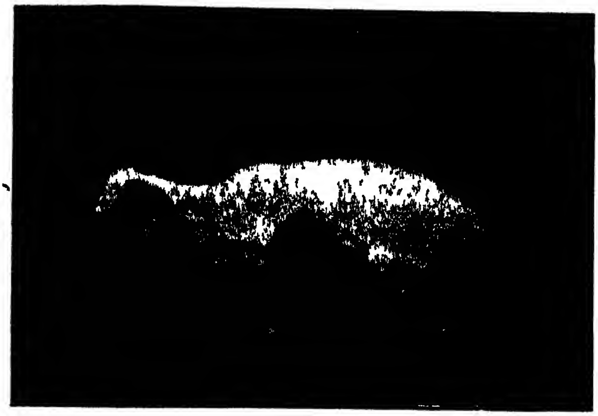

Fig. 9.—Sheep showing photosensitisation after injection of bile porhyrin.

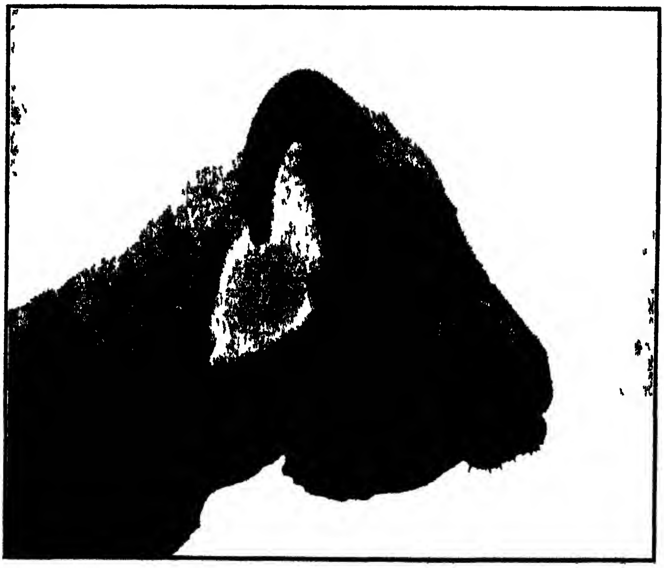

Fig. 10.—The same sheep 24 hours later.

Although the chemical identification seemed quite conclusive, a biological test was also carried out upon an authentic sample of phylloerythrin. This was prepared from chlorophyll by refluxing for 18 hours with 20% hydrochloric acid, decanting the dark blue-green liquid from tarry impurities, transferring the pigment to ether and purifying in the usual way. 2% and 5% hydrochloric acid extracted considerable quantities of pigment, but the bulk passed into 9% acid. In spite of frequent transferences, crystallisation of

this material was very poor. The spectrum was identical, however, with that of phylloerythrin and the methyl esters were similar. 41 mgm. of the free porphyrin were injected intravenously into a sheep. Well marked photosensitisation with the usual symptoms and sequelae was obtained, but there was no indication of any icterus.

The conclusion could thus safely be drawn that the photosensitising agent in geeldikkop is the pigment phylloerythrin. The significance of this discovery lies in the fact that phylloerythrin is a porphyrin of plant origin derived from chlorophyll. From the colouring matter of the Tribulus *plant, therefore, the photosensitising factor takes its origin.*

According to H. Fisher, the constitution of chlorophyll *a* and of phylloerythrin are to be expressed as follows:—

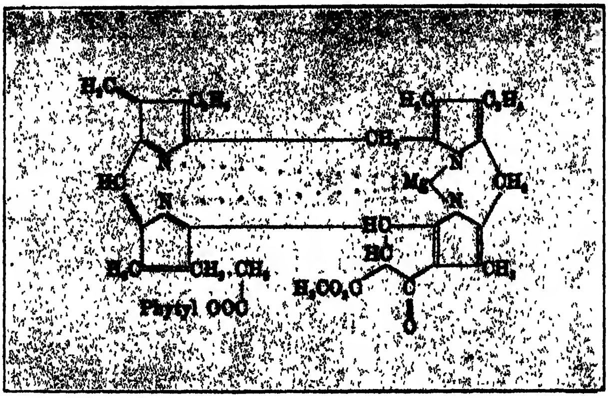

Fig. 11.—Chlorophyll.

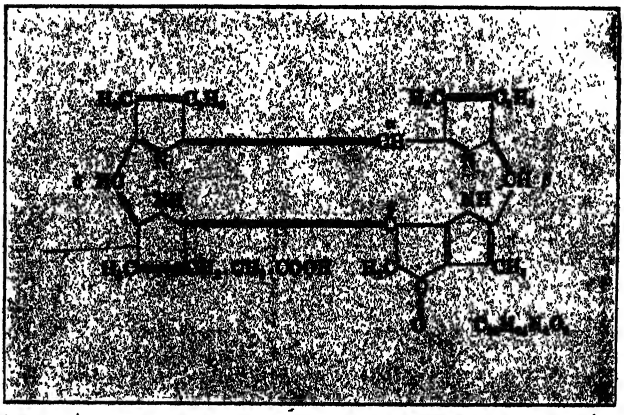

Fig. 12.—Phylloerythrin.

It will be seen that the production of the porphyrin necessitates only the removal of magnesium, saponification of the phytyl and methyl ester linkages and a simple decarboxylation of the chlorophyll molecule.

Phylloerythrin was first isolated from the faeces of herbivorous animals by Marchlewski (1904) who demonstrated that its excretion was related to the quantity of chlorophyll in the diet. This we have verified by our own experiments.

Marchlewski (1904-5) showed the identity of phylloerythrin with the pigment "bilipurpurin" detected in bile by Löbisch and Fischler (1904). Kemeri (1924) described a similar pigment in human faeces. Fischer and his collaborators (1931; 1931; 1932) were the first to show that phylloerythrin is a true porphyrin and to elucidate its chemical constitution.

With regard to the mechanism responsible for the biological formation of phylloerythrin from chlorophyll and the site of these reactions our knowledge is, at present, very meagre. The entire question of the metabolism of ingested chlorophyll by various animal species is, in fact, in a most unsatisfactory condition. Fischer and Hendschel (1932) have shown that caterpillars break down chlorophyll for the main part to a substance with the following constitution which they have named *phyllobomycin*.

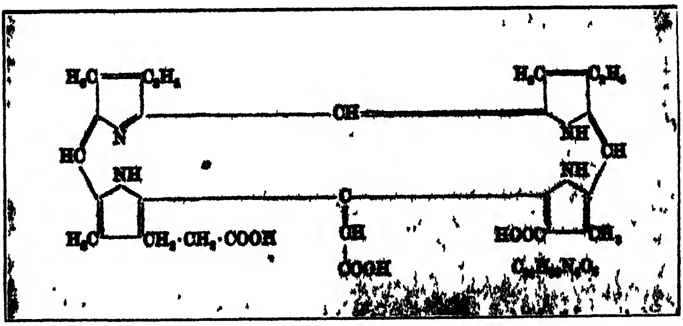

Fig. 13.—Phyllobombycin

Phylloerythrin is apparently not formed by caterpillars. In sheep's faeces, they were able to detect the presence of three closely similar pigments, the probophorbids α, β and γ, in addition to phylloerythrin.

During the course of our own work, which was more particularly concerned with the mechanism of phylloerythrin formation in the alimentary canal of the sheep, a communication appeared by Insmann and Rothemund (1932), in which they stated that traces of this pigment were to be found even in the rumen. Their experiments were qualitative and did not go far enough to permit of conclusions being drawn as to the agencies concerned in the formation of this pigment from chlorophyll.

We propose in a subsequent paper of this series to go more fully into the questions of chlorophyll metabolism in different animals, reproducing here only such data as is a necessary coadjunct to the experimental findings in the main enquiry as recorded above.

RELATION OF PHYLLOERYTHRIN EXCRETION IN FAECES AND BILE TO THE QUANTITY OF CHLOROPHYLL IN THE DIET.

As stated previously, an increase in the concentration of phylloerythrin in fistula bile was invariably noticed subsequent to the operation when the animals were liberally supplied with green stuff. In order to study this point more closely, a number of sheep were transferred to a diet consisting of coarse, yellow packing straw shown to be practically free from chlorophyll. The excretion of phylloerythrin in the faeces was followed by examining samples daily by the acetic acid-ether method. Within about a week, phylloerythrin and other chlorophyll derivatives were present only in traces. A fistula was then inserted into the gall bladder and after examining the bile for a few days, the animals were transferred to a chlorophyll-rich diet consisting of fresh, green lucerne or barley supplied *ad libitum*. Records were kept of the volume of bile secreted daily but these data along with other pertinent matter will be presented in a subsequent communication. Typical experiments are the following:—

Sheep 35287 previously maintained upon a chlorophyll-free diet was operated and a biliary fistula introduced.

Days after Operation.		*Units of Phylloerythrin in Bile.*
0		Nil
1		Nil
3		Nil
4, 5, 6	worked up together	Nil
7	put on to fresh green lucerne	0·2
8		6·5
9		12·4
10		13·0
11		9·5
12		9·5
13		13·2
14		14·2
15		16·2
17		21·6
18		25·0
19		32·0
22		32·4
23	fistula tube sloughed	—

Sheep 35290, companion to above, treated similarly:—

Days after Operation.		*Units of Phylloerythrin in Bile.*
0		Nil
4, 5, 6	worked up together	Nil
7	put on to fresh green lucerne	0·8
8		5·5
9		9·2
10		13·9
11		8·6
12		5·5
13		17·8
14	tube partially blocked by Stilesia worms: animal photosensitive.	

As was to be expected, it was found that sheep in which the bile duct had been ligated did not show any signs of photosensitivity so long as they were maintained on a chlorophyll-free diet, but did so within a short space of time when green lucerne was allowed.

The following two examples may be quoted:—

Sheep 35430 and 35422 maintained on chlorophyll-free diet, then operated and the bile duct ligatured. The bile taken from the gall bladders at the time of operation contained a trace only of phylloerythrin. They were exposed daily to the sun but evinced no sensitivity. After one week green lucerne was fed. Two days later sheep 35430 was markedly photosensitive and on the third day 35422 also became sensitive. Samples of faeces showed the presence of phylloerythrin and other chlorophyll derivatives.

Attention was next paid to the distribution of phylloerythrin in the various parts of the alimentary canal of sheep with a view to disclosing the mechanism of its formation. The methods employed were based upon the acetic acid-ether procedure, all values being related to the dry weight basis, since moisture content is a very important factor in quantitative data of this kind.

Individual sheep that had been maintained for some time upon a chlorophyll-rich diet were slaughtered and representative samples taken post-mortem from the contents of the rumen, omasum, abomasum, duodenum, jeunum, ileum, caecum, proximal, middle and distal portions of the large intestine.

The quantities of phylloerythrin found are expressed for convenience in " rumen units ", one rumen unit being equal to 10 of the bile units as previously defined.

A typical result is shown in Fig. 14, the phylloerythrin contents of the different parts of the alimentary canal being recorded graphically. It is evident that the primary seat of formation of this pigment is the rumen; some secondary formation may occur in the caecum and colon. It appears probable, as will be demonstrated in a subsequent communication, that the symbiotic micro-organisms inhabiting the alimentary canal are the agencies responsible for the formation of phylloerythrin.

SUMMARY.

The pigment responsible for the photosensitivity in geeldikkop and also that developing after operative ligature of the bile duct in sheep (experimental icterus) has been isolated and identified as phylloerythrin, a porphyrin derived from chlorophyll.

In the absence of chlorophyll from the diet, experimental animals neither became photosensitive nor could phylloerythrin be isolated from the bile, serum or faeces.

The agencies responsible for the biological transformation of chlorophyll into phylloerythrin in the sheep are under investigation. Preliminary results show that the phylloerythrin is formed in the fore-stomachs and is probably a product of protozoal or bacterial activity.

The icterogenic factor in geeldikkop is still under investigation.

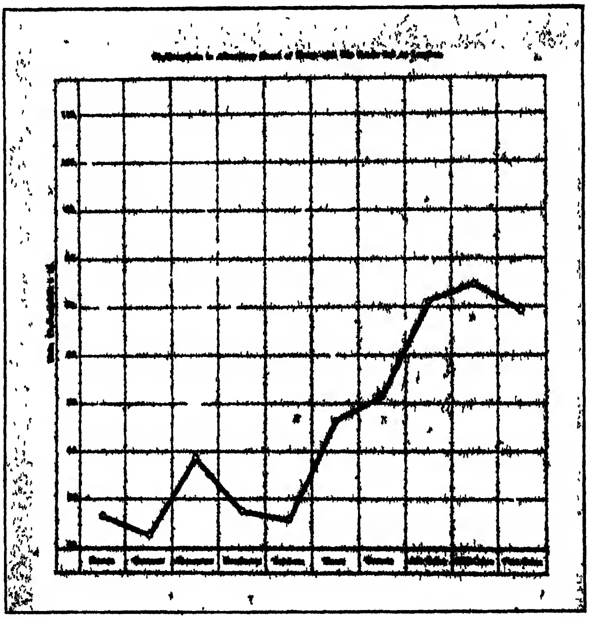

Fig. 14.—Phylloerythrin in alimentary canal of sheep with bile fistula fed on lucerne.

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Section VI.

Mineral Deficiency and Metabolism.

Onderstepoort Journal of Veterinary Science and Animal Industry, *Volume* 3, *Number* 1, *July*, 1934.

Recent Advances in the Chemistry of the *Plasma Proteins* and their Complexes.*

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CONTENTS.

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INTRODUCTION.

There is no department of physiological chemistry of more vital importance to the physiologist, the pathologist and the clinician than that of blood chemistry, consequently research upon the constituents of the blood is continuous and progressive year after year. New substances are continually being detected as normal or pathological constituents of cells or plasma, methods improved, various inter-relationships discovered, and the structure of well recognized constituents is being gradually more completely elucidated.

In any review of blood chemistry which is to be kept within reasonable proportions, some restriction of subject is imperative. In the following pages attention will be directed towards one particular department of blood chemistry in which our outlook has, in the past ten or fifteen years, suffered the most profound changes. It will not be possible to deal with fibrinogen and the mechanism of blood clotting, but an attempt will be made to summarize the modern conception of the individual identities and interrelationships of the remaining serum proteins and to discuss recent advances in the chemistry of the complexes which these proteins are known to form with lipoid and carbohydrate materials.

Any attempt at the inclusion of pathological data would open up so wide a field that it would be impossible in the present review to deal adequately with this phase of the subject. It is felt that by confining attention to normal materials and normal processes a more useful purpose will be served. For similar reasons no attempt will be made here to deal with recent advances in methods applied ro blood chemistry.

THE PROTEINS OF THE SERUM.

The earlier work at the beginning of the present century revealed the fact that normal serum contained about 7 per cent. of protein material amongst which could be distinguished an albumin and a globulin fraction. For a comprehensive review of the earlier work, reference may be made to Petschacher (1930). Hammarsten's (1902) article is a good presentation of the subject from the point of view prevailing at the close of the century before the classical work of Hardy (1905) upon the serum globulins. It is really Hardy's work which ushered in the new era culminating in the researches of Sørensen from the Carlsberg Laboratory and his theory of the constitution of proteins as reversibly dissociable compound systems (Sørensen 1930).

At the time that Panum (1851) showed that a precipitate of protein material was invariably formed when normal serum was diluted with water and slightly acidified or a stream of carbon dioxide gas passed through the diluted liquid, this was considered to be the only protein body of the serum. It was orginally called "casein" or "serum casein" by Panum (1851), but later designated "globulin" by Schmidt (1862), who considered it to be a single substance. Kühne (1868), however, was of the opinion that two different substances were produced according to the mode of preperation, by acidification with acetic acid or by carbon dioxide, and accordingly proposed the terms "paraglobulin" and "sodium

albuminate ". A return to the earlier views of Panum was, however, made by Weyl (1877), in whose opinion the precipitate consisted of a single substance which he named " serum globulin ".

The quantity of protein which separates from serum by the use of any of the above methods is only a little over 1 per cent. Actually much larger quantities are present. Hammarsten (1878) found that by saturation of the serum with magnesium chloride, no less than 63 per cent. of the total protein was precipitated, although this procedure was calculated to remove only globulins, leaving any albumin in solution. He therefore came to the conclusion that serum contained much greater quantities of " globulin " than had hitherto been supposed. Burckhardt, some years later, challenged this view on the grounds that when the precipitate obtained according to Hammarsten was redissolved in water and dialysed until salt free, only a fraction of it separated out, neither could further quantities be precipitated by acidification or treatment with carbon dioxide gas. It appeared to Burckhardt that Hammarsten's precipitate must consist of globulin admixed with albumin. It is of interest, in view of our later experience regarding the behaviour of globulin fractions on dialysis, to note that Hammarsten (1884) in reply argued that the presence in serum of other substances might greatly affect the solubilities of the protein constitutents and that by treatment of such a globulin fraction, not precipitable by dialysis, with sodium chloride after the manner of fractional precipitation, such interfering substances could be largely removed and a conversion of non-precipitable into precipitable globulin be brought about. He pointed out that only in solubility and temperature of coagulation did these substances appear to differ, but added, however, " weder die Indentität beider bewiesen, noch die Möglichkeit, dass der Niederschlag ein Gemenge von zwei oder mehereren Globulinen sei, in abrede gestellt sein soll ".

With the introduction of the technique, due to Hofmeister of salting out the different proteins by means of ammonium sulphate solutions of varying degrees of saturation, a big advance took place. Kauder (1886) was able to show that by precipitation of serum with ammonium sulphate up to half saturation, substantially the same material was precipitated as was thrown out by full saturation with magnesium chloride, as in Hammarsten's technique. As emphasized by Pick (1902), there is an interval of no precipitation from serum by ammonium sulphate, between the upper precipitation limits of the globulins and the lower limits of the albumins. From this time onwards, the separate individuality has been generally conceded of two main fractions of the serum proteins—albumins and globulins. With reference to the numerous attempts to prepare artificial globulins from albumin, compare the recent work of Hooker and Boyd (1933). Of course, chemical data, based upon analyses of the various fractions, furnish much more reliable evidence of similarity or dissimilarity than qualitative physical characteristics, such as those of solubility. Elementary analysis of protein materials is of limited help, however, on account of the small differences encountered in carbon, hydrogen and nitrogen content of various individuals. The content of sulphur or phosphorus is nevertheless frequently a valuable guide.

The following figures from Kestner (1925) illustrate the fact that the sulphur content of serum albumin is distinctly higher than that of the globulin fraction:

	C	H	N	S
Serum albumin cryst. ...	51·92–52·08	6·96–7·11	15·89–16·03	1·73–1·9
Serum albumin amorph. ...	53·04–53·5	6·75–7·1	15·71–16·04	1·77–2·31
Serum globulin	52·71	7·01	15·82–15·85	0·97–1·38

The difference in phosphorus content is even more pronounced. Sørensen (1930) has obtained purified serum albumins containing no phosphorus, whilst the globulin fractions all contained appreciable quantities of phosphorus, the proportion of which to nitrogen increased the less soluble the particular fraction was. Some pseudo-globulin preparations contained only minimal quantities of this element.

The difference in composition between (total) serum albumin and (total) serum globulin is still more clearly brought out by a comparison of the figures for the nitrogen distribution due to Hartley (1914) (see table 1), and those of amino-acid content cited from Kestner (see table 2). It will be noticed that glycocol is absent from serum albumin.

TABLE I.

	Total globulin. %	*Total albumin.* %
Ammonia N	7·7	5·8
Melanin N	2·0	1·1
Cystine N	2·0	3·5
Arginine N	10·9	10·4
Histidine N	6·3	6·7
Lysine N	9·0	16·3
Total basic N	28·2	36·9
Total filtrate N	62·0	56·5
Filtrate amino N	59·9	54·2
Filtrate non-amino N ...	2·2	2·3

TABLE II.

	Globulin.	*Albumin.*
Glycocol	3·5	absent
Alanine	2·2	2·7–4·19
Valine	2·0	present
Leucine	15·0–18·7	20·0–30·0
Aspartic acid	2·5	3·1–4·43
Glutamic acid	8·5	7·7
Proline	2·5–2·8	1·0–2·34
Tryptophane	4·0	1·4
Oxyproline		1·04
Phenylalanine	2·7–3·8	3·1–4·24
Tyrosine	2·5–6·6	2·1–5·8
Serine		0·56–0·6
Cystine	0·7–4·1	2·3–7·1
Histidine	0·8–1·7	2·2–3·72
Arginine	3·4–4·5	4·38–4·75
Lysine	4·6–6·8	7·48–11·29
Ammonia	1·75	0·95

A comparison of the ratio of total nitrogen to amino nitrogen titratable by formol was made by Obermeyer and Wilhelm (1912-1913) for serum albumin and globulin. Their figures were 14-10 for the former and 23-18 for the latter, which is in accordance with the fact that serum albumin contains the greater proportion of lysine.

Differences in specific refraction were found by Robertson (1912), who gave the figures for serum globulin 0·00229 and for serum albumin 0·00177.

Characteristic differences in the ultra-violet absorption spectrum have also been described by Dhéré and by Smith (1929), who proposes to determine the ratio of albumin to globulin in a mixture of the two by an analysis of the ultra-violet absorption spectrum.

Further confirmation of the individuality of the albumin and globulin fractions of serum is afforded by the immunological experiments of Dale and Hartley (1916), and of Doerr and Berger (1922). The former demonstrated the anaphylactogenic properties of these proteins and showed that the response of the sensitised animal was specific for the individual fraction with which it had been sensitised. Doerr and Berger further showed that, in the case of globulin sensitisation, the latent period is shorter and the resultant shock more intense than in the case of albumin. Berger has also pointed out that the increase of the one serum fraction in the organism and decrease of the other, are in no way related.

It would appear from an impartial appraisal of the various lines of evidence that there is good reason to believe that the albumin and globulin fractions produced in the ordinary way from serum represent chemically distinct proteins or associations of chemically similar proteins. The strongest argument in favour of this view is the demonstrable difference in composition between the materials. So little is known concerning the factors exciting biological sensitivity and formation of immune bodies, that the immunological evidence, at first sight so dramatically convincing, must be considered with great caution. This is all the more imperative, since Dale and Hartley (1916) found the euglobulin and pseudoglobulin fractions to exhibit a well-marked biological difference from which one would be tempted to draw the conclusion that here at least two distinct individual proteins were under consideration. The recent work of Sørensen (1925) however, makes it abundantly clear that the globulin complex consists not of two proteins but of a group of associated complexes whose individual components may be gradually and progressively dissociated by suitable treatment, a subject which will be more fully discussed under the heading of the serum globulins.

Association with other materials, differences in degree of dispersion, etc., may so affect the properties of an unstable colloid system such as a protein dispersion, that it is difficult to draw rigid conclusions from observed differences in precipitability by electrolytes, etc. The theory of salt precipitation advanced by Hafner and Kürthy (1924) and based upon relative polarisability may here be mentioned. The experiments of Adolf and Pauli (1924) should also receive attention, since these authors showed that, by electrodialysis, the serum proteins could be separated into an albumin

fraction, soluble in water and not precipitated by half saturation with ammonium sulphate and a globulin fraction which was completely insoluble in water.

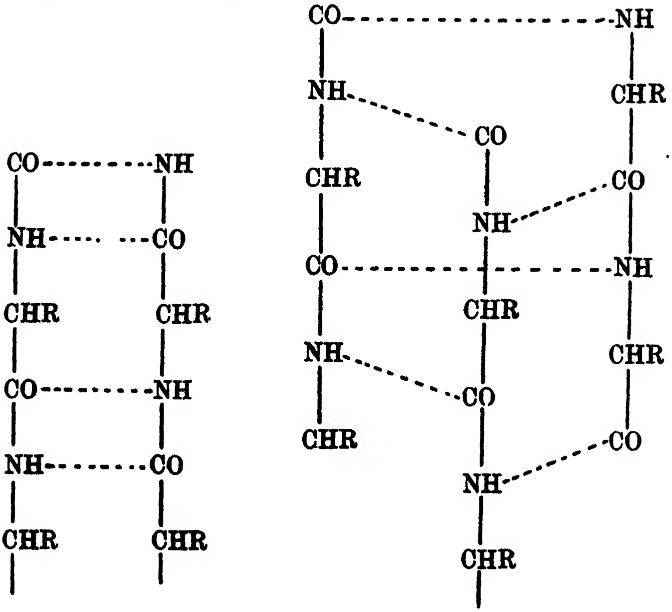
That there is a large difference in the degree of dispersion between the albumin and globulins in native serum seems to be certain from ultra-filtration and other experiments, e.g. Mutzenbecher (1931), also from Svedberg and Sjögren's (1928) researches upon the isolated proteins, but this difference in physical state with its consequent reflections upon chemical behaviour is just one of the reasons why great diffidence should be shown in coming to the conclusion that albumins and globulins are different in the sense, for example, that glucose and galactose are different. Is it not possible that in native serum we have a number of polypeptide associations (hauptvalenzketten) capable of grouping themselves into larger units by virtue of residual or secondary valencies, as is suggested by Sørensen in his theory of soluble proteins as reversibly dissociable compound systems, and that what really happens when the equilibrium is disturbed by the introduction of a quantity of an electrolyte, such as ammonium sulphate, is that those components with similar electro-chemical properties are forced into association and precipitate as a fraction to which we give the name albumin or globulin, as the case may be?

If such a protein precipitate represented a single, chemically pure substance, its physical properties should remain constant, such as, for example, its solubility in salt solutions of given concentration and hydrogen ion activity. That this is not so in the case of crystallized serum albumin, has been shown by Sørensen (1930), so we are forced to the conclusion that even crystallinity, in the case of these complex systems, is no criterion of purity. Sørensen's experimental data will be considered later on.

It may be as well to digress somewhat at this point in order to consider Svedberg's results, more particularly as they affect the serum proteins and decide what weight should be given to such evidence in coming to a decision regarding protein individuality.

Svedberg and his associates (1930) have applied the ultracentrifuge to measure the molecular complexity of proteins in solution. In general, it was found that all proteins exhibited a molecular weight which was a multiple of a common factor 34,500. A small group possessed much larger molecular weights, but the others fell regularly into classes of 1, 2, 3 or 6 times 34,500. In many cases the values so obtained checked well with those derived from such direct measurements as those of osmotic pressure, as in the case of egg albumin, investigated by Sørensen. Svedberg also showed that each protein possessed a comparatively small range of pH on each side of its iso-electric point within which its molecular weight, as measured by the ultra-centrifuge, remained unchanged. In the case of the 2, 3 and 6 multiple groups, however, cautious regulation of pH resulted in a dissociation of the protein into components of the lower orders. Outside this range again irreversible dissociations or decompositions took place. Svedberg's brilliant results at once call for two explanations, why the basic figure of 34,500 for all proteins and why only the 1, 2, 3 and 6 multiples, never 4 and 5?

A solution to these two problems has been suggested by Astbury and Woods (1931). In the first place it is assumed that the figure 34,500 is a statistical average, for which there is some evidence, and that actually polypeptide chains could be produced of indefinite length and weight were it not for the instability caused by atomic vibrations. It is this disruptive vibration which is assumed to limit the length of the chain to a unit having the approximate molecular weight 34,500. One would like to see quantitative evidence advanced in support of this hypothesis, against which, however, nothing definite will be said at the moment. The second question, once the answer to the first is conceded, can, according to Astbury and Woods, be solved by application of the elementary principles of crystalography. A polypeptide chain can form an association with another by virtue of the residual attractions resident in its $-NH_2$ and $>CO$ groups. Three combinations, and three only, are then possible, involving 2, 3 or 6 chains, as follows:



as in b, with each chain replaced by a pair a.

a b c

The four classes of proteins having complexities represented by 1, 2, 3 and 6 times 34,500 can thus be explained.

It is very important to note that in some cases Svedberg found that a certain minimal concentration of protein was necessary before homogeneity of particle size was obtained. This is significant in view of the fact that Sørensen has demonstrated that in systems such as gliadin, dissociation of the components occurs only when the protein concentration is very low. As previously pointed out (Rimington, 1931), the dominance of the figure 34,500 throughout all Svedberg's results must point to some uniformity of pattern common to all natural proteins, but it is not certain that this represents the lowest unit out of which the more complex structures are built. A protein which under Svedberg's ultra-centrifugal

conditions would appear to be homogeneuous can, by Sørensen's comparatively simple technique of applying the elementary physicochemical principles of solubility, be shown to be divisible into dissimilar portions.

THE ALBUMIN FRACTION.

Doubts as to the homogeneity of the serum alubumin fraction have not been wanting. Thus Halliburton (1884) on the grounds of differences in coagulation temperature distinguished at least three albumins in serum, α-, β- and γ-albumin, which he considered to be separate proteins. The first, α-serum albumin, coagulated at 70-73°, β-serum albumin at 76-78°, and the γ-albumin at 82-85°. It is, however, very questionable whether these temperature differences are sufficient to warrant the existence of three distinct proteins. Even Halliburton's (1886) own results would seem to indicate that other factors may influence the behaviour of the proteins in a given serum. Thus in many cold-blooded animals the coagulation temperature indicated that only γ-serum albumin was present, whilst in eel blood α- and β- and in avian and mammalian bloods all three α-, β- and γ-albumins appeared to be present. These anomalies are somewhat difficult to understand. Even more forceful arguments have been put forward based upon the finding that in no case can the entire albumin of serum be obtained in the crystalline condition, a non-crystallizable portion invariably remains in greater or lesser proportion. In fact, it is a matter of the greatest difficulty to get any albumin crystals at all from the serum of such animals as guinea pigs, cats and bovines (Gruzewska, 1899). As previously pointed out, however, crystallisability is no guarantee of the purity of a particular protein. Wichmann (1898) drew attention to the fact that when a mixture of serum albumin and egg albumin or of these two with lactalbumin is treated with ammonium sulphate in the usual way, the crystals which form convey the impression of perfect homogeneity

Sørensen's (1930) more recent researches have established beyond doubt that apparently homogeneous crystalline serum albumin is in reality an association of mutually interacting component systems having different solubilities, etc. His evidence will now be considered in detail.

Experiments were conducted with crystalline serum albumin made by the method of Hopkins and Pinkus (1898) both from native serum and containing about 0·3 to 1·5 mg. P per gm. N and also with practically phosphorus-free preparations obtained from serum protein powder according to the Hardy-Young (1910, 1922) alcohol technique, or the Hewitt (1927) alcohol-ether method. No appreciable difference in behaviour was found between these preparations, thus indicating that the attachment of lipoids cannot be alone responsible for the irregularities in solubility observed. The crude Hopkins-Pinkus preparation, when subjected to the first recrystallization, deposited a lipoid-containing, greenish, amorphous substance.

In every series of experiments all factors except those intentionally varied were kept strictly under control.

A well-marked dependence of solubility upon the total quantity of protein present in the system was demonstrated, employing three fractions T_1, T_3 and M_5 of a crystalline serum albumin. These designations were arrived at in the following way: To the protein solution sufficient ammonium sulphate solution was added to throw down a slightly soluble fraction T_1. As the salt concentration was increased the main bulk of the albumin separated in a middle fraction M, the filtrate containing easily soluble L_1. T_1 and L_1 were recrystallized and the mother liquor from the first and precipitate from the second added jointly to M_1, which was now subjected afresh to the entire scheme of fractionation yielding T_2, M_2 and L_2. This process was repeated five times.

Fig. 1 represents the results graphically, *S* being the protein hydrate in gm. per 100 gm. of water found dissolved at ammonium sulphate concentration *S* where *Z* was the quantity of protein hydrate present in the experimental mixture. [For experimental details, etc., see Sørensen C.R. Lab. Carlsberg 16, No. 12, p. 17 (1927).]

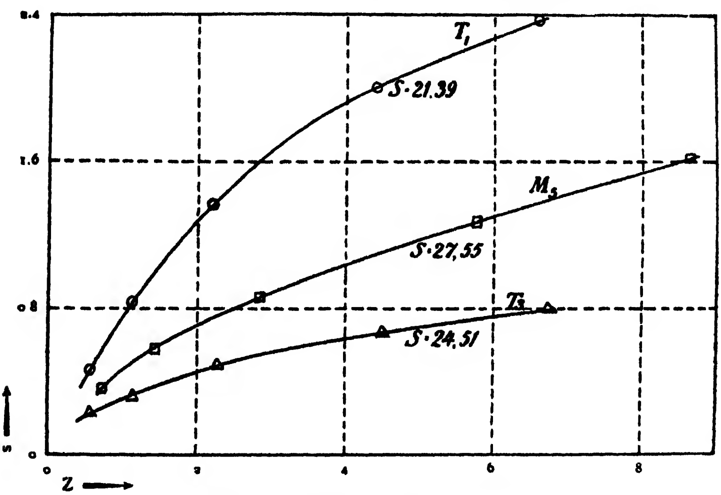


Fig 1. (from Sørensen, 1930).

Again it was shown by selecting particular fractions and determining their solubility under strictly controlled conditions that under identical circumstances their individual solubilities varied widely, also the solubilities at varying pH as is shown by Fig. 2. The fractions designated A_I, A_{II} and A_{III} were the sparingly, moderately and easily soluble fractions of a phosphorus-free albumin preparation and were obtained by crystallization to ammonium sulphate concentrations of (40), (47) and (60) respectively in Sørensen's notation. T_1C was gained by recrystallizing T_1 three times; T_3b and M_5c are to be similarly interpreted.

Such striking differences in solubility behaviour would suggest that there might be found underlying differences of a chemical nature in the various fractions, indicating the separation of a heterogenous mixture of proteins or, possibly, actual decomposition, carried to a greater or lesser degree, of the original albumin material.

The results of Sørensen's analyses did show that quite marked differences in composition existed between the various fractions, but only certain easily determined amino-acids were estimated and the experimental evidence was insufficient to reveal any connection between solubility and composition.

The figures quoted below illustrate this fact:—

	T_3b	L_2f	L
Humin N	0·02	0·39	0·04
Ammonia N	5·80	7·16	5·97
Cystine N	4·38	3·65	4·57
Tryptophane N	0·66	2·10	0·91

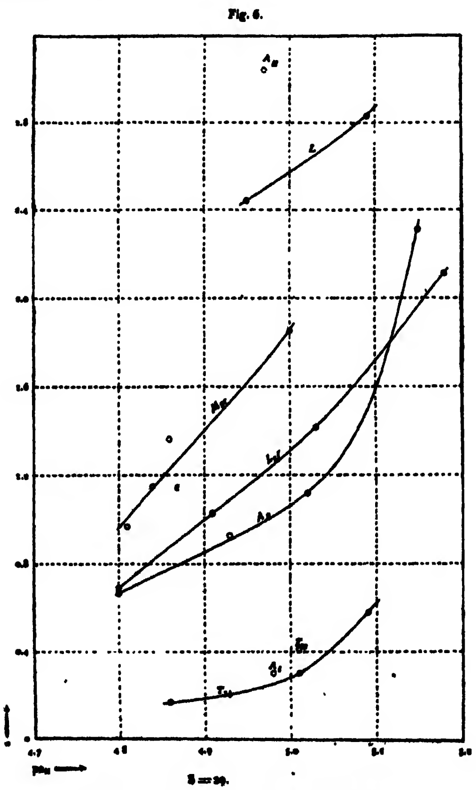


Fig. 3 (from Sørensen, 1930).

As was earlier pointed out, T_3b represents a twice recrystallized sparingly-soluble fraction, whilst L and L_2f represent fractions at the other extreme, characterized by great solubility. It is evident that T_3b and L do not differ markedly in composition. L_2f on the other hand shows that by successive recrystallizations of the more soluble L fractions, a product pronouncedly richer in tryptophane N and in humin N accumulates.

Determinations of the acid- and base-binding capacities showed, on the other hand, that under equal conditions all fractions were alike, as were their optical rotations. Previous work (Sørensen,

1924) had shown that crystalline fractions of widely differing solubilities all exhibited the same osmotic pressure. These facts would seem to disprove any suggestion that processes of decomposition or denaturation had taken place.

As Sørensen himself remarks, the chemical analysis of the various fractions was not detailed enough to reveal the connection which must undoubtedly exist between solubility and chemical composition. In view of the significant figure for humin N in the fraction L_2f quoted in the table and the demonstration by Rimington (1929, 1931) that both serum albumin and serum globulin contain quite considerable quantities (about 1 per cent. in the former and 3·8 per cent. in the latter) of a complex polysaccharide as an integral part of their molecules, also the work of Lustig and his associates (1931) upon the carbohydrate content of various serum globulin fractions, it would be of the greatest interest to examine whether solubility of the various serum albumin fractions can be related directly to their carbohydrate contents. The experiments of M. Sørensen and Haugaard (1933) would seem to suggest that this may be so (see section on bound carbohydrate of blood).

THE GLOBULIN FRACTION.

As has already been pointed out, the addition of acid or CO_2 gas to diluted serum results in the precipitation of only about one-sixth of the total quantity of protein; by salting out with magnesium chloride or half saturation with ammonium sulphate, a further quantity precipitates, representing about another 62 per cent. of the total. Hammarsten regarded both fractions as globulin, a view which was strongly contested by Burckhardt, who showed that reprecipitation was incomplete after the fraction was subjected to dialysis. Marcus (1899) confirmed these findings, but pointed out that in other respects the materials—the water soluble and water insoluble—were closely similar. Hammarsten (1884), whilst not denying the possible association of two or more globulins in the precipitate, demonstrated that their solubilities were largely affected by the presence of impurities which he succeeded in removing by sodium chloride precipitation.

The duality of the globulins was clearly shown by Fuld and Spiro (1901-2), who found that the rennetic ferment and anti-rennetic substance of serum could be separated by fractional precipitation of the globulin fraction with ammonium sulphate. The former appeared in the 28-33 per cent. fraction, the latter in that precipitated between 43 and 46 per cent. saturation. Full and Spiro first applied the terms euglobulin and pseudoglobulin, which are still in use to-day.

Pick (1902) considers that there are no sharp limits of precipitation for these two substances, but that they precipitate reciprocally. That some such phenomenon occurs appears also to be borne out from experience with antitoxins and immune bodies in different animal species (Pick, 1902), Belfanti and Carboné, Freund and Joachim (1902), Marcus (1899), Dieudonné (1897), etc., also by the fact that by often-repeated dialysis and resolution, it is impossible to obtain a pseudoglobulin which is completely soluble in water.

Adolf and Pauli's (1924) electrodialysis method does seem capable, however, of effecting a division into completely soluble and completely insoluble globulin.

For an explanation of this peculiar behaviour it is natural to turn at first to the possibility of associated substances, such as lipoids, affecting the solubilities of the priteins. An association of the globulins with lipoids was early recognized and many studies have been made with the object of elucidating the nature of the combination. Thus Chick (1914) thought it not improbable " that euglobulin in serum is a complex material formed from pseudoglobulin by association with some serum lipoid to the presence of which it owes its phosphorus content "; the process could even be pictured as a gradual and progressive one.

With reference to the nature of protein-lipoid associations, more will be said in a later section, but from Sørensen's (1930) results it would appear that a part at least of the phosphorus of eu- and possibly also of pseudoglobulin is an integral part of the protein and independent of lipoid material.

As in the case of serum albumin, Sørensen's (1925, 1926, 1930) researches from the Carlsberg Laboratory have done much to clear our views concerning the individuality of the two serum globulins. In brief, his conclusion is that one is here dealing with an association of mutually interacting complexes the conditions of whose solubility, association or dissociation are governed by factors such as the concentration of the solution, the presence or absence of electrolytes, etc. In the first series of experiments upon serum globulin, Sørensen (1925) used preparations made by fractionation of the diluted serum with ammonium sulphate, subsequent dialysis, etc. The proteins so obtained contained fairly large amounts of phosphorus, whereas the later preparations which he employed and which were made from " serum powder ", the total protein obtained from serum by the use of alcohol or ether-alcohol at low temperature, contained considerably less phosphorus as the following figures show. There was no significant difference, however, in behaviour between the proteins of the former or latter series:

	mg. coagulable P/gm. protein N	
	From crude serum.	From serum powder.
Pseudoglobulin	0·5–3·0	0·1–0·4
Euglobulin	2 – 40	0·1–0·4

An euglobulin preparation, four times reprecipitated by ammonium sulphate, collecting only the material thrown out at concentrations of the salt up to (30), was subjected to dialysis and the precipitate and residual solution worked up separately. The precipitate, after washing, was dissolved in 0·6 N KCl and the solution then diluted to 0·03 N whereby a part of the protein was precipitated. This was again dissolved in 0·6 N KCl and the mixture diluted to 0·1 N. A further separation into soluble and insoluble fractions occurred. The quantities of protein remaining in the first and second KCl mother liquors were dialysed until salt-free but only a portion was precipitated. These processes were shown to be capable of indefinite repetition. There is thus a continuous and progressive dissociation or " scaling off " of pseudoglobulin

particles from the complex originally present. Similarly it was shown that, by suitable treatment, the more easily soluble pseudoglobulin fraction could be made to yield certain quantities of euglobulin. Sørensen denotes the relationship as follows:—

$$E_p\, P_q \rightleftharpoons a\, Px + \ldots\ldots\ldots + b\, En\, Pm + \ldots\ldots\ldots + E_r\, P_s$$

where E denotes an euglobulin and P a pseudoglobulin complex or molecule and

$$p = bn + \ldots\ldots\ldots\ldots\ r$$
$$q = ax + \ldots\ldots\ldots\ldots\ bm + \ldots\ldots\ldots\ldots + s$$

Turning now to the solubility relationships at different salt concentations, it was again found, as in the case of serum albumin, that the solubility depended upon the total quantity of protein added to the system, the exact behaviour being also related to the degree of previous fractionation to which the samples had been subjected. Thus, only by very prolonged fractionation and washing could an euglobulin be obtained which exhibited a fairly constant solubility in 0·02N NaCl. When stronger salt solutions were used as solvent for this material, dissociation of pseudoglobulin complexes again took place and the solubility of the material was, consequently, once more dependent upon the total amount of protein in the system. The stronger salt solutions have a more potent effect in bringing about dissociation.

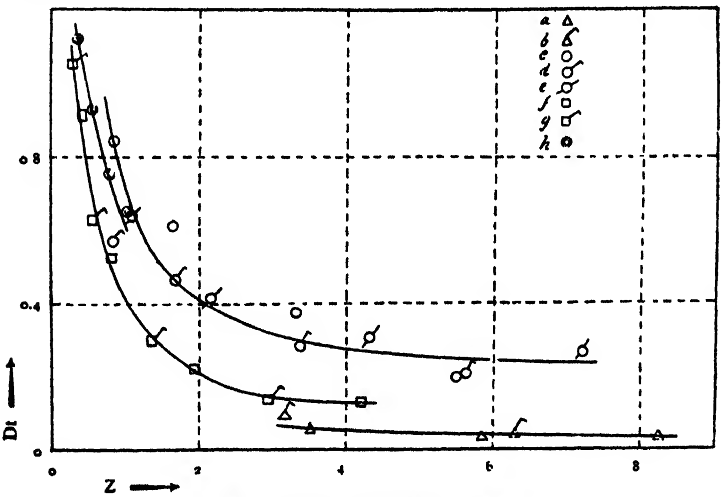


Fig. 3 (from Sørensen, 1930).
a and *b*: egg albumin; *c d*, and *e*: serum albumin; *f* and *g*: pseudoglobulin; *h*: euglobulin.

Reciprocal action between precipitate and dissolved complexes was clearly shown by Sørensen to occur in these systems.

It must be concluded that by these means it is impossible to obtain either pure euglobulin or pure pseudoglobulin, all preparations being complexes whose solubility rises in proportion to the quantity of pseudoglobulin they contain. It is of interest as a close to this section to reproduce a figure from Sørensen's work illustrating the dissociation tendency (Dt) of the various proteins he has studied as a function of their concentration (Z).

BEHAVIOUR OF THE SERUM LIPOIDS.

As has been mentioned earlier, the precipitate produced by adding an equal volume of saturated ammonium sulphate to serum, contains quite considerable quantities of lipoid material as does also the first crude deposit of crystalline albumin. Upon recrystallization of the latter, most of the accompanying lipoids are left behind in the form of a greenish insoluble residue (cf. Troensegaard and Koudahl, 1926). It is probable that one is dealing with a mechanical admixture or loose association between protein and lipoid rather than true chemical combination, since there seems to be no regularity in the relative proportions of the two constituents. However, the fact that serum is normally clear and translucent requires an explanation. By shaking serum with ether only minimal quantities of lipoid material are removed which would suggest that some mutual interaction must exist between these substances. Addition of ammonium sulphate or other electrolytes suffices to break down this equilibrium so that the two materials are easily separated. Treatment with alcohol or ether-alcohol in the cold gives rise to protein preparations which are practically lipoid-free, so that here again conditions must have been disturbed.

It is noteworthy that in Sørensen's (1930) experience, all attempts to restore the combination of protein and lipoid have failed to reproduce the original clear serum. Fairly stable emulsions could be formed but these were always opalescent.

A few years ago, Mâcheboeuf (1927, 1929) subjected the problem of the serum lipoids to a fresh investigation and claimed to have isolated a true protein-lipoid combination in a yield of about 2·5 gm. per litre from horse blood. The globulins were first precipitated by ammonium sulphate and the filtrate acidified to pH 3·8 which caused a voluminous precipitate to settle. This was dissolved in water to which sufficient ammonia was added to bring the pH to 7. Solution and reprecipitation at pH 3·8 was repeated 10 times, by which time the composition of the substance was said to become constant.

It was an association of 50 per cent. protein, 23 per cent. lecithin and about 18 per cent of cholesterol esters, and dissolved in water at neutral or alkaline reaction to produce perfectly clear solutions from which the lipoids could not be removed by shaking with ether.

On extracting crude serum with cold alcohol or ether-alcohol as in the preparation of " serum powder ", one would expect to find Mâcheboeuf's substance in the mother liquors, but as Sørensen (1930) shows, its detection in these is not realisable in practice. One must conclude that on evaporation of the solvent cleavage of the compound takes place.

More recently Theorell (1930) has subjected the whole question of the serum lipoids and their relationship to the proteins to one of the most searching investigations of the subject so far performed. In addition, his paper may be referred to for a critical review of the previous literature on the subject. As he justly states, far too little attention has been paid to the purity of the materials used in

attempts to reconstitute stable lipoid-protein emulsions resembling serum. Thus, ordinary cholesterol is fairly readily emulsified in water by suitable technique, but this property is lost on repeated recrystallization. Theorell describes in detail the special procedure by which he was able to obtain 0·7 to 0·85 per cent. emulsions of pure cholesterol.

When such an emulsion is added to fresh serum a portion of the cholesterol is taken up in clear solution and the sedimentation velocity of erythrocytes is found to be markedly retarded thereby. At the same time the resistance to hypotonic salt solutions (fragility test) is increased. It appears that a part of the dissolved cholesterol is taken up—possibly adsorbed by the cells.

Examination of such a choloesterized serum shows that the precipitation limits of the proteins towards ammonium sulphate have not altered, but that the main quantity of the added cholesterol falls with the globulin fraction. Fibrinogen, in the case of cholesterised plasma, carries down little. In a similar way it was shown that when lecithin sols are added to serum or plasma and the excess centrifuged off, a certain quantity has gone into clear solution in the liquid, whilst the excess has removed with it a fraction of the cholesterol originally present. Lecithin, as does cholesterol, causes a diminution of sedimentation velocity but acts antagonistically to cholesterol in that it decreases the resistance to hypotonic sodium chloride solutions. A part of the added lecithin appears to be adsorbed by the corpuscles, although no alteration in electric charge of the latter can be detected.

On salting out with ammonium sulphate, the precipitation limits of the proteins are found to be unaffected. A part of the excess lecithin is carried down by the fibrinogen, the remainder by the globulin fraction.

For the sake of comparison with the above findings Theorell (1930) prepared an euglobulin fraction from horse serum by electrodialysis in a Pauli apparatus and also the total globulin and albumin fractions from the same serum by ammonium sulphate precipitation. All these protein preparations were analysed for cholesterol and lecithin (i.e. Phosphorus x 25) with the following results, from which it will be seen that the quotient cholesterol/lecithin falls progressively from euglobulin to albumin:—

Protein fraction.	Cholesterin / lecithin.	Cholesterin / protein.	Lecithin / protein.
Total protein	0·51	0·013	0·0255
Euglobulin	0·79	0·023	0·0292
Pseudoglobulin plus albumin (=dialysate filtrate) ...	0·45	0·011	0·0246
Total globulin	0·75	0·011	0·0153
Albumin	0·41	0·015	0·0366

The effect of the pH upon the distribution of the plasma lipoids among the various protein fractions was also studied. The results may be presented summarily in the following table, although in attempting to interpret these figures it is important to note that

altering the pH towards the acid side causes a progressive co-precipitation of the less highly dispersed fractions at the usual salt concentrations, and in all probability also an alteration in the equilibrium between free and protein-bound lipoid:—

Protein fraction	pH	$\frac{\text{Cholesterin}}{\text{Lecithin}}$ for "Fibrinogen."	"Globulin."	"Albumin."
I	7·46–7·41	∞	1·08	0·50
II	6·54–6·42	∞	1·05	0·50
III	5·99–6·12	∞	1·20	0·52
IV	5·57–5·59	1·44	0·71	0·59
V	5·06–5·02	1·00	0·66	0·73
VI	4·70–4·77	0·54	0·66	0·80

A general comparison of the data for sedimentation velocity with total cholesterol content fails to reveal any correlation, although it would appear to be certain that this phenomenon is largely influenced by the lipoids of the plasma. The existence of both free and protein-bound cholesterol in serum was made very probable by the work of Handovsky, Lohmann and Bosse (1925), who also found a correlation, slight in degree, between the euglobulin content and the ether-extractable cholesterol. Theorell, in the work under discussion, by

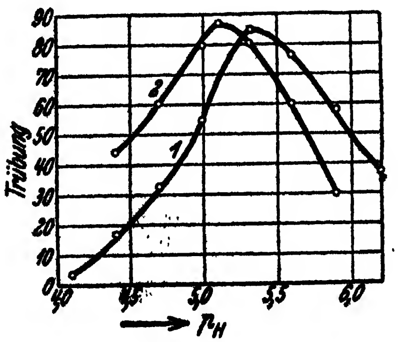


Fig. 4

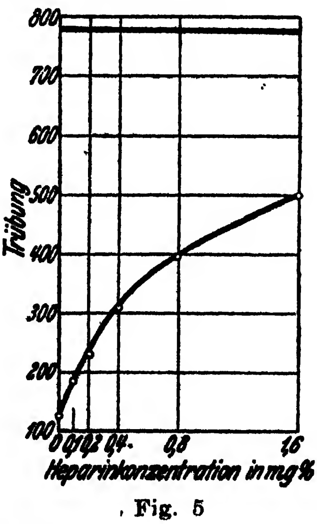


Fig. 5

(from Schmitz and Fischer, 1933, A).

means of ether extraction experiments at different pH values of the serum and of varying duration, establishes quite clearly the fact that a part of the total cholesterol is easily extracted at any pH and is probably, therefore, free cholesterol in simple, colloidal solution, whilst the remainder is only extracted with difficulty and shows a well-marked pH effect, being most easily extractable by ether at pH 5·5–6·0, a reaction suggestively close to the iso-electric points of fibrinogen and the globulins. A reversible association between cholesterol and protein was successfully demonstrated with isolated protein fractions. Only at still more acid reactions than pH 5·5 do albumin and cholesterol form associations.

As a result of a large number of extraction and sedimentation velocity experiments upon a large number of sera, the hypothesis was substantiated that only the cholesterol not in combination with fibrinogen or globulin exerts an inhibitory effect upon sedimentation. A correlation coefficient of $-0 \cdot 63 \pm 0 \cdot 10$ was found between the free cholesterol and sedimentation velocity.

The effect upon sedimentation of heating plasma is to be associated with alterations in the colloidal conditions and is independent of the lipoids.

Schmitz and Fischer (1933, A, B) distinguish between the effect of H ions upon the serum proteins (acid reaction) and that of salts, particularly ammonium sulphate (salt reaction), and show how these two factors operating together may be made to indicate the lability of the colloidal protein system.

Lipoids such as heparin were shown by Fischer (1932) to increase the acid reaction, the flocculation optimum being displaced towards the acid side, they act, therefore, in the sense of stabilisers of the serum proteins. This effect with a mixture in their original proportions of albumin and globulin isolated from serum, is well brought out in Fig. 4.

pH 5 was chosen as a suitable value at which to make comparative determinations of turbidity and the effect of the progressive addition of heparin to such a protein system upon the turbidity at pH 5 is illustrated in fig. 5. It will be noticed that the salt reaction remains uninfluenced.

When now the results are so expressed as in Fig. 6 plotting the difference between the salt reaction and acid reaction D as ordinate against the logarithm of the concentration of stabilisor (heparin), log S, a linear relationship is found to hold, i.e. the stability decreases regularly as the logarithm of the stabilisor increases.

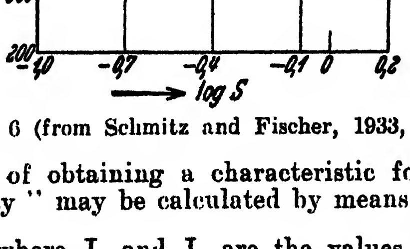


Fig. 6 (from Schmitz and Fischer, 1933, A).

For the sake of obtaining a characteristic for any serum the "degree of lability" may be calculated by means of the expression $L = 100 - \frac{100 \times J_1}{J_2}$, where J_1 and J_2 are the values for the acid and salt reaction respectively. Log L then gives the "degree of lability" of the serum.

No reference has as yet been made to the work of Brinkman and Van Dam (1920) and Brinkman and Wastl (1921), to whom many of the early results in this field were due. In particular, attention will here be called to their demonstration of the effect of diet upon red cell fragility, haemolysis and regeneration. The feeding of lecithin caused a well-marked intravital haemolysis in the rabbit with increased red cell fragility. Lecithin in the diet was also found to be essential for normal blood cell regeneration.

Adopting a procedure of progressive precipitation by ammonium sulphate, Lustig and Katz (1930) were able to show that the protein fractions brought down from normal serum at concentrations of 33 per cent. ("euglobulin"), 50 per cent ("pseudoglobulin"), 50-60 per cent. ("albumin I"), 60-66 per cent. ("albumin II"), 66-75 per cent. ("albumin III") and then separated according to solubilities in water, sodium chloride, sodium carbonate and sodium hydroxide solutions (Freund and Joachim, 1902), differed from each other in such respects as total nitrogen content, number of amino and of carboxyl groups, etc. In a further communication the same authors (1931) have investigated the lipoid contents of the various ox serum protein sub-fractions. Lustig and Botstiber (1930) had already shown that the lipoids of human euglobulin, pseudoglobulin and albumin fractions were characteristic for each protein, and Lustig and Katz extend this specificity to the sub-fractions also. The highest lecithin content was found in the sodium chloride-soluble sub-fractions of the eu- and pseudoglobulins, the lowest in the water soluble sub-fractions. Their table of results is reproduced below:—

Distribution of lipoids in the sub-fractions of serum proteins.

Proteins	Lipoids in 100 g. protein %	Lecithin in 100 gm. protein	Lecithin in % of total lipoid.	Cholesterin in 100 gm. protein.	Cholesterin in % of total lipoid.
Euglobulin water sol. ...	0·56	0·11	19·7	0·12	21·4
Eug. NaCl sol.	2·33	0·41	17·6	0·48	20·6
Eug. Na_2CO_3 sol.	1·66	0·46	28·3	0·41	24·7
Euglobulin NaOH sol.	9·70	1·98	20·4	2·95	30·4
Pseudoglobulin water sol. ...	3·32	1·08	33·4	0·92	27·7
Pseudoglobulin NaCl sol. ...	3·29	1·35	41·0	0·92	28·0
Albumin I ...	3·15	1·18	37·8	1·02	32·3
Albumin II ...	6·26	2·82	45·0	2·10	33·5
Albumin III...	4·86	1·53	31·6	2·13	44·0
Total proteins of ox serum ...	4·75	1·44	30·3	1·61	33·9

The immunological behaviour of the various serum sub-fractions has been the subject of additional communications by Freund and Lustig (1932) and Lustig and Katz (1932). The relative proportions and chemical characteristics, NH_2– and COOH groups, total N and carbohydrate contents of the sub-fractions in such pathological conditions as cirrhosis, sarcoma and carcinoma, have been investigated by Lustig (1931) with interesting results.

"BOUND SUGAR" OF THE BLOOD AND PROTEIN-CARBOHYDRATE COMPLEXES.

In a recent review of 250 pages supplemented by over 500 references, Grevenstuk (1929) subjected to a detailed, critical examination the experimental results and claims of those who have investigated the so-called "bound sugar" or "protein sugar" of the blood. No attempt will be made, therefore, to traverse this field again from its historical aspect, since Grevenstuk's paper is readily available; the issue will be narrowed down, rather, to a discussion of the occurrence of carbohydrate groups as an integral part of the serum proteins.

The fact that the reducing power of whole blood is increased by acid hydrolysis is an incontestable fact, but to assume, as have most workers in this field, that the increase in reducing power is due to the liberation of *glucose*, previously held in combination with the *protein*, is quite unwarrantable. In order to avoid the prejudice of terms, Grevenstuk in the monograph referred to alludes to the precursor of the extra reduction as the "hydrolysable substance", "Hy–S", thus avoiding any implication as to its nature. His discussion suffers, however, in another respect, that of engendering in the mind of the reader, unintentionally, the idea that the same Hy–S may be found in the plasma, corpuscles and possibly also in the serum proteins. In any approach to the subject, the very reverse should be uppermost in the mind. The methods employed by different investigators should be critically compared before endeavouring to appraise the value of their respective results, which so often stand in mutual contradiction. Grevenstuk has performed a signal service in reproducing, in all essential detail, the procedures adopted by the workers of the French and Italian schools, especially are the methods of deproteinization employed of the greatest significance.

Let us now clarify the problem. As stated above, the fact is undisputed that, when whole blood is subjected to acid hydrolysis, the reducing power of the deproteinized filtrate is found to be greater than that prior to hydrolysis. The general concensus of opinion is also to the effect that plasma behaves similarly. With regard to the proteins, we have conflicting results.

Now, plasma or serum contains substances, some of which are known to liberate reducing bodies on boiling with acids or to increase in reducing power such as, for example, hexose phosphates, glycuronates, creatin, etc. It is of interest that with regard to the latter, Benedict found that by heating a solution with n – HCl at 117° for 30 minutes, the change from creatin to creatinine, with higher reducing power, was practically complete. The conditions are almost identical with those used by the French workers in their "bound-sugar" determinations (Bierry and Fandard, 1918).

Turning next to the deproteinizing agent, it is well known that some mixtures precipitate protein fission products less completely than others and differences in final reducing power have in fact been found according to whether, for example, mercuric nitrate (Patein-Dufau) or sodium tungstate (Folin) mixture was employed for the

removal of the protein cleavage products formed as a result of the treatment with acid. The use of the Hagedorn-Jensen method where zinc hydroxide is the deproteinizing agent, as in the experiments of Bigwood and Wuillot (1927, A, B, C) is quite unpermissable!

Not only is there a danger of incomplete removal of interfering protein bodies, however, there is also the chance that particular deproteinizing agents might remove one or other of the actual carbohydrate substances it is desired to estimate, a point which has been most persistently and unaccountably overlooked. In 1929 Dische pointed out that mercuric nitrate and alkali, as used by Bierry and his collaborators, removed some constituent from the hydrolysate which in view of the writer's own, later experiments, was almost certainly glucosamine. Thus the figures obtained by the French school of workers are, probably, without exception, too low.

Fontès and Thivolle (1927, A, B) found that deproteinization of blood with mercuric nitrate led to lower reducing values than when sodium tungstate was used, but also pointed out that if such a tungstate-deproteinized blood filtrate was hydrolyzed with acid for half an hour and then treated with mercuric nitrate and the reducing power again determined on this filtrate, it was found to be unchanged. Grevenstuk comments upon this finding as follows: "Blut enhalt eine Substanz, die nach Hydrolyse ebenso stark reduziert wie zuvor (also nicht zur Hy–S gehort), aber vor Hydrolyse wohl, danach aber nicht mehr von Mercurinitrat gefallt wird!"

The facts allow of another interpretation. Suppose there is present in the tungstate filtrate a carbohydrate complex represented by G–M where M possesses a free reducing group. G–M is supposed to be removed from solution by mercuric nitrate and alkali by virtue of groupings in the component G, but to remain unprecipitated by sodium tungstate. The higher reducing power of a tungstate filtrate follows at once. Now suppose such a filtrate is hydrolyzed, G–M being split into its components and the mercuric nitrate reagent added. G will in all probability be precipitated and M remain behind in solution. The filtrate from this last precipitation would then exhibit a total reduction equal to that of the tungstate blood filtrate but greater, by M, than that of an original mercuric nitrate blood filtrate.

Apart from the points afore mentioned, the method of direct acid hydrolysis possesses other serious drawbacks which may be enumerated below as follows:—

(*a*) If the period of heating be too short, or the temperature not sufficiently high, hydrolysis may be incomplete and the reducing value found, therefore, too low.

(*b*) Even boiling pure sugar solutions with acids leads to a certain amount of destruction the degree of which varies both with the particular carbohydrate, the kind of acid, its concentration and the temperature and duration of heating, so that it is quite impossible to make any quantitative allowance for this effect [Pavy (1896), Krok (1918), Glaessmann (1926), and unpublished results of the writer].

(*c*) In the presence of certain amino-acids or protein breakdown products and hot, strong acids, as in the usual condition of hydrolysis, carbohydrates readily condense with the nitrogenous substances to form " humin ", a dark amorphous material devoid of reducing properties. Gortner (1916) and his associates have shown that tryptophane is the amino-acid chiefly responsible and in unpublished experiments the writer has found that when tryptophane and glucose in the molecular proportions 2:1 are refluxed in 20 per cent. hydrochloric acid for 24 hours, the formation of humin is nearly quantitative. Glucosamine reacts to only a very slight extent under these conditions.

Clearly, it must be accepted as a foregone conclusion that when whole blood, or even the isolated blood proteins, are autoclaved with acids and reducing power determinations carried out, the quantity of sugar found will be considerably less, to a variable extent, than that which was actually formed. Had any of the methods in use been tested by a simple recovery experiment, this would at once have been obvious.

Considering the unsatisfactory state of the whole problem of " bound " or " protein-sugar ", the writer some years ago endeavoured to find a solution to one aspect at least, whether purified serum proteins contained any carbohydrate material as an integral part of their molecule—by the only method possible, namely, that of isolating the carbohydrate constituent in a state of purity and determining its chemical structure.

It is surprising that previous investigators have given so little forethought to the efficient purification of their starting material and to the proper choice of hydrolytic agent. Mineral acids have been almost exclusively employed in spite of the obvious objections to their use outlined above. Thus both Krawkow (1896) and Langstein (1902) claimed to have isolated an osazone from acid-hydrolyzed serum albumin and the latter author identified glucosamine among the reaction products by the method of benzoylation.

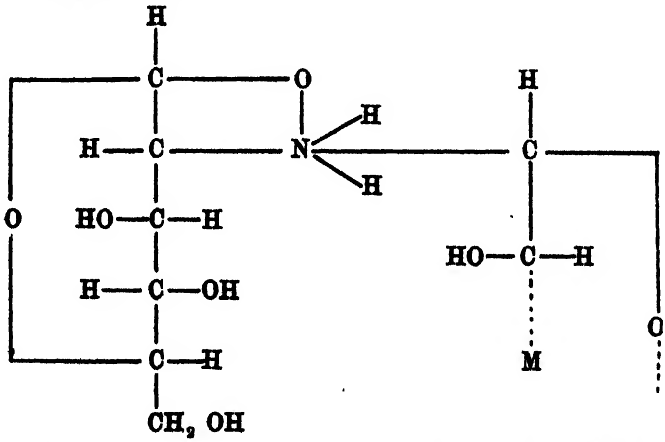
Somewhat similar results were obtained in the case of serum globulin by Krawkow (1896), Eichholz (1898), Abderhalden et al. (1904), Langstein (1903, 1905, 1906), Condorelli (1924, A; 1926) and others. Their individual findings were, however, far from being in good agreement, and no clear insight was obtained into the nature of the " bound sugar ".

In the writer's own work (Rimington, 1929; 1931) attention was especially paid to the purification of the serum protein fractions used and an alkaline hydrolyzing agent, barium hydroxide was employed, this having the advantage that the carbohydrate complex was fairly readily liberated from its attachment to the protein without in itself suffering much resolution into its components. The greater stability of glucosides towards alkalis than towards acids is well known. The same complex was also obtained after digesting the protein with trypsin. Its isolation was achieved as follows (Rimington, 1931):—

The purified protein, serum albumin or globulin, was refluxed with 10 per cent. baryta for 36 hours, barium removed as sulphate and the carbohydrate precipitated by basic lead acetate and baryta,

the process being controlled by Molisch tests upon the filtrate. After washing, reprecipitation, etc., carbon dioxide was passed through a suspension of the precipitate in water until all carbohydrate material was liberated. Protein decomposition products were then removed by acid mercuric sulphate, keeping the H_2SO_4 concentration below 5 per cent., and after removal of Hg, Ba and SO_4 ions, the solution concentrated in vacuo to a syrup. This was mixed with dry methyl alcohol and the carbohydrate precipitated by addition of ether. It was purified by frequent reprecipitation and then dried in vacuo over sulphuric acid.

The substance so obtained contained nitrogen, and it was at first thought that the percentage of this element became constant at 4·1 per cent. However, such preparations always contained a little chlorine and it was subsequently found that this was due to the carbohydrate complex being incompletely freed from histidine, to which it appears to be bound in the protein molecule. More prolonged hydrolysis with baryta gave a substance, no longer giving a positive diazo reaction (Pauli's test) and free from chlorine, with a constant nitrogen content of 2·78 per cent.



This carbohydrate was found to be a complex of glucosamine (1 mol.) and mannose (2 mols.), most probably polymerized. No other sugar could be identified. It does not exhibit reducing properties until after acid hydrolysis, which liberates the individual components, and was optically inactive. It was not attacked by enzyme preparations. In order to account satisfactorily for its chief properties, the following structural formula was tentatively suggested for this carbohydrate; detailed evidence is presented in the writer's first paper. M represents the second molecule of mannose.

The same complex was present in both albumin and globulin, but the quantity was much greater in the latter.

In the light of these findings, many of the observations of former investigators fell into their true perspective. Thus the isolation of glucosamine from acid hydrolysates is readily understandable; also the fact that Dische (1929) found mannose to be present in an acid hydrolysate of whole plasma.

Many of the claims of Bierry, it is true, are at variance with the above results. Thus he (1928) failed to find any mannose in one careful experiment with an acid hydrolysate but isolated glucosazone and consequently attributed the whole of the extra reducing power to glucose, although, in a later publication he (1931) considered the presence of mannose, galactose and glucose to be probable.

As before mentioned, the chances of isolating, with certainty, any of the carbohydrate constituents from an *acid* hydrolysate of blood or plasma are remote except in the case of the comparatively stable glucosamine, but this, of course, yields the same osazone as glucose.

It is of interest to note, in passing, that Fränkel and Jellinek (1927) obtained a nitrogen-containing polysaccharide from egg-albumin and Levense and Mori (1929) later obtained this substance in pure form and showed it to be glucosamino-dimannose. They consider, however, that it was probably derived from ovomucoid adhering to their protein preparations since with recrystallization the carbohydrate-content of egg albumin decreases.

This interpretation was strongly criticized by the writer, who pointed out that, by repeated recrystallization a fractionation of the protein, in the Sørensen sense, was really achieved. It was suggested that the carbohydrate content of the individual components of the blood serum proteins might vary quite considerably, thus, perhaps, conferring upon them their respective solubility characteristics (see previous discussion).

A fractionation of this sort has quite recently been carried out in Sørensen's laboratory by M. Sørensen and Haugaard (1933), who actually found a very marked difference between the carbohydrate content of a readily soluble, crystalline serum albumin fraction (0·47 per cent.) and a sparingly soluble (also crystalline) fraction which contained not more than a trace (0·009 to 0·017 per cent.). These values were obtained by a colorimetric method.

Various methods have been proposed for the colorimetric determination of sugars in complex mixtures, glucosides or proteins. These are reviewed by Dische (1931). The writer, in his study of the serum proteins (1931) used both the methods proposed by Dische and Popper (1926), and by Tillmans and Philippi (1929) based upon colour reactions with indole and with orcinol respectively. Neither gives any colour with glucosamine but mannose, according to the indole method, develops only 65-70 per cent. of the colour of an equivalent quantity of glucose, whereas with orcinol it reacts quantitatively. The polysaccharide isolated from the serum proteins gave, as would be expected from a consideration of the above facts, 66·6 per cent. of the colour of an equal weight of glucose by the orcinol method and only 40 per cent. according to the indole method.

Serum globulin gave 1·468 per cent. of carbohydrate reckoned as glucose by the Dische-Popper method, and 2·279 per cent. by that of Tillmans and Philippi. A very carefully purified preparation afforded a figure of 1·3 per cent. as glucose (Dische-Popper) corresponding, therefore, to 3·7 per centfl of total carbohcdrate-glucose aminodimannose.

When examined in the same way, serum albumin was found to be much less rich in carbohydrate groups. Sørensen and Haugaard (1933) made a thorough study of the conditions under which the Tillmans-Philippi method could be carried out to best advantage and found that, by suitable adjustments, characteristic differences in the behaviour of the individual hexoses were observed such as to make it possible to identify them in mixtures, or in combination in more complex polysaccharides. In this they had recourse to measurements of colour absorption by the " step-photometer " and by selecting the two spectral filters 43 and 53 and plotting the ratio of the extinctions as measured by these, $\frac{E\ 43}{E\ 53}$, against the time of heating of the reaction mixture were able to obtain their basic reference curves for each sugar. Since the individual components of a polysaccharide contributed their effects additatively, the constitution of the latter could be " deduced " by finding the combination best superposed upon the experimental $\frac{E\ 43}{E\ 53}$ time curve yielded by the substance in question.

From the results of their experiments, they conclude that not only mannose but also galactose is present in serum albumin and globulin preparations, glucosamine, of course, gives no colour with the reagent and would not be detected by this method.

Whilst these findings must undoubtedly be looked upon as suggestive and significant, no certain conclusion can be drawn until galactose has actually been isolated from these proteins and fully identified.

The quantity of carbohydrate found by Sørensen and Haugaard in serum globulin was 1·82 per cent., as glucose, which as they show is equivalent to 3·41 per cent.* of a glucosaminodimannose or glocusamino-galacto-mannose in good agreement with the figure of 3·7 per cent. obtained by the writer. Attention must also be drawn to the work of Lustig and Haas (1931) who, using the Tillmans-Philippi method as originally described, showed that the carbohydrate content of their serum proteins was not altered by prolonged dialysis or repeated reprecipitation from alkaline solutions, after denaturation, by the addition of acid. Further, they investigated the carbohydrate content of the various sub-fractions of ox serum protein and found very marked differences. The following table is taken from their work:—

Protein sub-fraction.	*Carbohydrate content %.*
Euglobulin water—soluble	0·84
Euglobulin NaCl—soluble	0·98
Euglobulin Na_2CO_3—soluble	2·38
Euglobulin NaOH—soluble	8·50
Pseudoglobulin water—soluble	0·98
Pseudoglobulin NaCl—soluble	0·64
Pseudoglobulin Na_2CO_3—soluble	6·40
Pseudoglobulin NaOH—soluble	7·03
Albumin I	0·47
Albumin II	0·55
Albumin III	0·65

It is clear from the small difference in carbohydrate content between albumins I, II and III compared with the large difference found by Sørensen and Haugaard (1933) between that of their easily soluble and sparingly soluble albumin fractions, that the different procedures adopted do not effect the same type of separation.

Some space has been devoted to the consideration of "bound" or "protein" sugar in blood since it is felt that there are already sufficient indications in the literature to show that it may prove to be of distinct importance not only in the normal physiology of carbohydrate metabolism but also in pathological conditions. Much of the existing work, it is true, is marred by the uncertainty as to what substances were actually included in the determination and by the unreliability of the methods employed. The proper basis for future investigations has now been supplied, however, with the recognition of the nature of the carbohydrate and methods for its accurate determination.

No attempt will be made to discuss the various contributions to the physiology of bound sugar as most are referred to in Grevenstuk's (1929) article. Attention will only be drawn to the papers of Glassmann (1926), Freund (1885) and the Italian workers, especially Condorelli (1924), B; 1924, C), and to point out that Bordet's (1922) argument is not necessarily sound. He claimed that the bound carbohydrate of blood could not be regarded as attached to the proteins since Bierry's quotient $\frac{\text{Protein N}}{\text{“Protein sugar”}}$ is by no means constant, at least not in pathological cases. It was not appreciated, apparently, that different serum protein fractions might have widely differing carbohydrate contents [compare the table from Lustig and Haas (1931), quoted above] and thus an increase in the relative quantity of any one, considerably alter the $\frac{\text{Protein N}}{\text{“Protein sugar”}}$ quotient. Especially is it likely that such conditions might arise in pathological sera.

As the writer has previously pointed out (Rimington, 1929), the question whether the carbohydrate complex of the serum proteins takes a part in immunological phenomena is a pertinent one and should be explored by experimental investigation. Heidelberger (1927) and his associates have demonstrated the peculiar rôle played in this connection by the polysaccharides, some nitrogen-containing, of various strains of pneumococcus. Other examples have since been brought forward and the name "haptene" has been proposed for this class of specifically active substance. The demonstration that the serum protein polysaccharide is incapable in itself, of provoking antibody formation, does not exclude its possible function in a manner analogous with that of the haptenes. What grouping is responsible for the biological specificity of proteins is still unsolved. Possibly the phenomenon of specificity and antigenicity resembles that of enzymic action in requiring a specifically active centre situated on a colloidal carrier, the dependence for the manifestation being mutual.

It is significant that Avery, Goebel and Barbers (1932) have demonstrated that the synthetic gluco-proteins formed by coupling globulin with $\alpha-$ and $\beta-$p$-$aminophenol glucosides (by diazotization and mixing in alkaline solution) are not only antigenic but exhibit a specificity very similar to that encountered in the case of pneumococcus type II and Friedländer Bacillus type B.

Thus, whilst addition of the homologous glucoside to its antiserum completely inhibits the precipitins for both homologous and heterologous test antigens, the heterologous glucoside inhibits only for heterologous antigen having but slight effect upon the antibodies reactive with the homologous antigen.

In the previous study, made by Avery and Goebel (1929), where a gluco-globulin and galacto-globulin were employed, that is to say, antigens made by coupling globulin with the p-aminophenol glycosides of glucose and of galactose respectively and differing therefore only by the relative positions of one H and OH group in the sugar residue, a much stricter degree of specificity was found to obtain, each developing an antibody specific for the gluco-protein which had induced its formation. Moreover, the specific rôle of the sugar radical was demonstrated by the fact that the glycosides alone, unattached to protein, were capable of inhibiting specifically the precipitin reaction between the corresponding antiserum and homologous antigen.

Before leaving the subject of "bound sugar", mention must be made of the third protein of the serum, the so-called serum mucoid. Surprisingly little attention has been paid to this substance, the most complete study being that of Bywaters (1909), who also proposed a method for its determination (1906-7). Eichholz (1898) reported the isolation of a considerable quantity of an osozone melting at 204° from the products of its hydrolysis and Zanetti (1903) the identification of glucosamine as (tetra) benzoylglucosamine.

Bywaters, as a result of his study came to the conclusion that serum mucoid was a true protein, closely resembling ovomucoid, and not merely a proteose or protein split-product, as some had supposed on account of its not being coagulated by heating its solutions to boiling. He also determined its carbohydrate content by the yield of reducing substances after acid hydrolysis to be of the order of 24 per cent. Glucosamine was identified by the isolation of pentabenzoylglucosamine from such hydrolysates. Bywaters considered that the quantity of serum mucoid, normally about 0·3 to 0·9 gm. per litre of blood, varied markedly in response to various physiological conditions such as hunger, starvation, etc.

As a result of unpublished experiments carried out some years ago, the writer is able to substantiate Bywater's claims on the chemical side.

The mucoid was prepared from ox or sheep serum after first removing the albumin and globulin by heating, by evaporating in vacuo to a small volume, dialyzing thoroughly and then precipitating by addition of alcohol. The crude material was several times redissolved in warm water, the solution filtered and poured into about four volumes of alcohol. A second dialysis was also usually included.

It was obtained as a white powder, soluble in warm water and giving most of the usual protein colour reactions, with the exception, however, of the labile sulphur test, although analysis showed it to contain about 1·7 per cent. S. Fehlings solution was not reduced on boiling with a solution of the mucoid, but the Molisch reaction was strongly positive. Analyses of different preparations gave figures similar to those quoted by Bywaters for his own and Zanetti's preparations, thus:—

	C	H	N	S
Ox serum mucoid	47·43	7·23	12·31	
Sheep serum mucoid	47·78	7·13	12·75	0·91
Mean of Bywaters preparations	47·62	6·85	11·59	1·8
Zanetti's preparations	47·6	7·1	12·9	2·2

The carbohydrate content was determined by the method of Tillmanns and Philippi (1929). Expressing the results as glucosaminodimannose it approached 22 per cent., but some variation was noticed, possibly due to irregularities of the method (see Sørensen and Haugaard, 1933). The isolation of the carbohydrate complex has not yet been undertaken, but it is hoped to do this and to complete the study of this interesting protein in the near future when it will be seen whether the same carbohydrate grouping is present in the mucoid as in the other serum proteins.

That serum mucoid does exist in blood and is a distinct protein seems to be incontestable. It would be of interest to examine its fluctuations in various pathological conditions. Lewis and Wells (1926) have examined the immunological behaviour of both serum- and ovo-mucoids of different species and their results still further support the view that these proteins are distinct individuals. Thus serum mucoid was found to be antigenic, that of the sheep being more active (anaphylactic shock method) than that of the ox. No protection was afforded by the heterologous protein. Compliment fixation tests also showed a fairly high degree of specificity. Dog and sheep serum mucoids were shown by the uterine strip method to be distinct from each other and from the ovomucoids.

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A Routine Method for the Determination of Soluble Ash in Plant Material.

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THE growing prominence of pasture studies in agricultural research in this country has resulted in a great extension of the work reported on by du Toit and collaborators (1932). With a limited staff it became more imperative to reduce the time factor wherever possible in all methods employed for the analysis of samples of natural pasturage, without, however, unduly sacrificing accuracy. Although all the more important inorganic constituents had been determined in the past it was thought advisable to include soluble ash or silica-free ash for future work. Generally a figure for total ash is included with the mineral analysis of feeds and fodders. But when results for the total ash are given they also include silica which is not considered an essential element in nutrition, although it is present in considerable quantities in the ash of plant material.

A. Principle of the Method.

The ash of the herbage is treated with concentrated hydrochloric acid on the steam-bath so as to convert all the bases into chlorides, decompose the silicates and render the silica insoluble. The soluble salts are leached out with dilute hydrochloric acid and the soluble ash determined by difference in weight of the original ash before and after leaching.

B. Description of Method.

Approximately 6 gm. of finely ground material in a platinum dish of known weight are dried for at least three hours at 103° C. After 20 minutes in the desiccator the dish and contents are weighed on a " Bunge-quick-precision " balance and ignited in an electric muffle for 2 hours at about 450° C. The dish is then transferred to a desiccator and weighed after 20 minutes. The ash is moistened with distilled water and 3 c.c. concentrated HCl added. The acid is evaporated on the steam-bath and the ash allowed to dry for another hour on the steam-bath, whereby the silica derived from the decomposition of the plant silicates by concentrated HCl is rendered insoluble. 20 c.c. distilled water and 2 c.c. concentrated HCl are then added and the contents heated for a few minutes on the steam-bath, allowed to cool and filtered quantitatively through Whatman No. 40 filter paper into a 100 c.c. measuring flask. The filter is carefully washed with distilled water up to the mark on the flask. This filtrate of a 100 c.c. is used for the determination of Ca, Mg, K and Na. The insoluble residue, consisting of silica, carbon and often sand contaminating the sample, is washed back into the platinum dish with distilled water, evaporated to dryness on the

steam-bath, transferred to the electric muffle to dry for another 15 minutes at 300-350° C., allowed to cool in a desiccator and weighed. The difference between this weight and that for total ash gives the weight for soluble ash.

C. Experimental.

(a) Decomposition of Plant Silicates and rendering Silica Insoluble.

In order to test the reliability of the process of decomposing silicates in the ash of plant material with concentrated HCl and of the procedure for rendering the silica precipitated insoluble in the routine procedure described above, results thus obtained were compared with the figures obtained by treating the ash of the same specimen according to the method employed by Treadwell and Hall (1924) for the analysis of silicates decomposed by acids. Briefly, the powdered silicate is decomposed with 50 c.c. 3 N.HCl on the steam-bath, evaporated and heated for at least one hour at 120° C. in a closet to dehydrate the silica. The insoluble residues were in each case dried at 300-350° C. In the table below average percentages obtained for the soluble ash of each of three different plant specimens are presented.

TABLE 1.

Plant Specimen.	Soluble Ash. Treadwell & Hall.	Soluble Ash. Routine Method.
1. Green grass....	6 32	6 24
2. Green grass..........	6·90	6 86
3. Bush....................	7·10	7 07

(b) Drying the Insoluble Residue.

According to Mellor (1922) silica retains about 3 per cent. of water when dried at 300° C., the anhydrous compound being obtained only when heated to 500° C. Admittedly, then, the silica in the insoluble residue is not completely dehydrated in the routine procedure described. However, a more serious error is introduced by simply igniting the insoluble residue together with the filter at 500° C. and computing the soluble ash figure from the weight of the residue thus obtained and the weight of the original ash, because after the soluble material has been leached out any residual carbonaceous material will readily ignite at 500° C. In the case of old mature grasses and the leaves of bushes a negligible amount of carbon is left over in the ash when ignited for 2 hours at 450° C. However, this does not apply to green grasses. Even if ignited at higher temperatures where there is danger of volatilizing alkali chlorides, etc., a considerable amount of carbon remains with the ash. This carbon does not, however, seriously interfere with the quantitative extraction of the soluble ash as is apparent from Table 3 further on in this paper, but, if the insoluble residue is ignited at 500° C. this residual carbon will be included with the figure for soluble ash.

The temperature to which charcoal has been heated and its resultant physical condition largely determines the temperature at which it will ignite. Consequently, in the case under consideration it may be assumed that a negligible amount of the residual carbon will ignite if heated for 15 minutes at 350° C., the upper limit for drying the insoluble residue.

Soluble ash figures obtained by drying the insoluble residue at 300-350° C. as described in the routine method and by igniting the insoluble residue together with the filter at 500° C. are tabulated below. Column 3 gives figures for residual carbon calculated as percentages of the original plant material and obtained by treating the ash of the same specimens according to the method for determining carbon, sand and silica of the Association of Official Agricultural Chemists (1920). Column 4 gives the difference between columns 2 and 3. An error which is negligible for routine purposes is indicated by these differences representing true percentages soluble ash when the insoluble residue has been dried at 500° C.

TABLE 2.

	Per cent. Sol. Ash Insol. res. dried at 300–350° C.	Per cent. Sol. Ash Insol. Res. ignited at 500° C.	Per cent. Residual Carbon.	Column 2 minus Column 3.
1	6·383	7·105	0·620	6·485
2	6·889	7·239	0·338	6·901

(c) Recovery of Soluble Ash.

Davidson (1931) found that appreciable amounts of inorganic constituents remain behind in the insoluble residue after the soluble fraction of the ash of plant material has been leached out with dilute hydrochloric acid. To overcome this difficulty he resorted to volatilization of the silica with hydrofluoric and sulphuric acids prior to leaching out with dilute HCl. The majority of pasture samples coming in from the veld are, however, more or less contaminated with sand, and while every effort is being made to remove as much of this sand as possible, it is impossible in many cases to remove the finely-divided particles of dust adhering to the plant material. If silica is volatilized from the ash of such specimens the results for soluble ash and consequently for some of the individual constituents will be too high, because of the soluble bases derived from the decomposition of the sand dust contaminating the samples.

On the other hand, it is freely admitted that some of the inorganic constituents will be too high when the extract has been prepared from plant material contaminated with sand dust. Woodman *et al* (1926) introduced a correction for soil included in their pasture cuts by analysing a sample of such soil, calculating the soil content of the grass from a knowledge of the silica content of the grass as cut and the soil-free grass and then making the necessary corrections for the figures for individual constituents.

Such a procedure is obviously impracticable where thousands of pasture samples from all over the country have to be dealt with at one central station. At the same time, if the figures for individual inorganic constituents as otbained by Woodman for worm casts can be taken as a criterion, and if, prior to grinding, pasture samples are handled in such a way as to reduce adhering soil particles to a minimum (3 per cent. and less), the effect of soil contamination on the analytical results will be insignificant.

Without volatilizing silica hydrochloric acid extracts of insoluble residues after they had been ignited have, however, been prepared and some of the constituents determined. In Table 3 below the soluble ash figures from first and second extractions together with values for some individual constituents in first and second extracts are tabulated. Values for silica are included and all figures are given as percentages of the original dry plant material.

TABLE 3.

	Extraction.	Soluble Ash.	P_2O_5.	CaO.	K_2O.	Na_2O.	Silica.
1	1st......	14·43	0·1179	5·552	—	2·220	1·44
	2nd.....	0·08	0·0006	0·032	—	0·034	
2	1st......	6·20	0·20	1·02	2·51	0·33	5·57
	2nd.....	0·094	0·002	0·007	0·014	0·002	
3	1st......	6·89	0·41	0·76	3·56	0·17	4·30
	2nd.....	0·059	0·003	0·005	0·002	0·001	
4	1st......	4·56	0·970	1·00	0·71	1·08	4·25
	2nd.....	0·047	0·003	0·005	0·0158	0·026	
5	1st......	6·40	0·972	1·60	1·98	0·028	3·56
	2nd.....	0·072	0·005	0·007	0·0036	trace	

A consideration of the figures in Table 3 proves conclusively that for routine purposes the method for preparing the extracts for the determination of inorganic constituents in plant ash as described in this paper is highly satisfactory. The error introduced by ignoring the additional quantities of individual constituents yielded by a second extraction of the insoluble residue is negligible.

D. Concluding Remarks.

The nature and quantity of ground plant material incinerated for the preparation of the extract and the determination of the soluble ash largely determine the efficiency with which the acid-soluble fraction of the ash is recovered by filtering and washing to 100 c.c. A small quantity of material means less salts to be washed from the filter and this in turn will ensure a more thorough washing with the limited volume of wash water. (See Treadwell and Hall.)

An additional advantage in using about 6 gm. instead of approximately 10 gm. of material as directed by Malan and van der Lingen (1931) is that larger aliquots of the extract may be used for the determination of sodium, calcium and magnesium. It was pointed out by Louw (1933) that the concentration of potassium in a plant extract limits the aliquot allowable for the direct determination of sodium. In some cases aliquots as low as 0·2 c.c. had to be used. Although the actual amount of sodium present in a larger aliquot from the extract prepared by incinerating about 6 gm. material will be the same, the possible error through pipetting very small volumes is eliminated. With regard to the micro-determination of calcium and magnesium the actual concentration of these constituents in the extract limits the volume of the aliquot permissable. When about 10 gm. of plant material is used for the preparation of the extract it will often be necessary to dilute the extract before a suitable aliquot can be taken, whereas with the smaller quantity (6 gm.) of herbage preliminary dilutions of the extract will seldom, if ever, be necessary.

E. Summary.

(1) A routine method for the determination of soluble ash in plant material is described in detail.

(2) Evidence is presented to show that the results obtained by this rapid method compare very favourably with those obtained by following the standard procedure.

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* It is stated in the 1930 edition of "Methods of Analysis" (p. 102), which came to hand after the completion of this work, that the alkali soluble silica of plants is rendered insoluble by heating the ash for one hour on the steam-bath after it has been treated with concentrated hydrochloric acid, a procedure similar to that described in this paper.

Section VII.

Animal Husbandry.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 3, Number 1, July, 1934.

The Influence of Feed on the Merino Sheep.

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1. INTRODUCTION.

Feed plays an important part in wool production. The sheep farmer is aware that during droughts his sheep are lighter in body weight, and produce lighter and finer fleeces. Different research workers have concluded that feed influences the sheep and its fleece.

Probst (1926) found that the variation of wool growth along a staple from one of his stud rams could be explained by the influence of winter feed conditions.

Wilson (1927) in dealing with wool production in California asserts that " sheep which are improperly or poorly fed produce light frowsy fleeces lacking the life which is so essential to good wool."

Duerden and Bosman (1927) found an absence of uniformity of growth along a wool staple and it was presumed to have resulted from feed influences.

Hardy and Tennyson (1930) in describing wool fineness influenced by rate of growth, showed that the period of greater growth was associated with a general thrifty condition of the sheep, also that the weight of wool increased with an increase in length and fibre thickness.

Wilson (1931) found considerable differences in the fleece characteristics when three Romney wethers were fed on maintenance rations and then on sub-maintenance rations.

Weber (1932) found a difference in scoured fleece weights, fibre fineness and length by feeding different quantities of ground corn and alfalfa to sheep.

Snell (1932) found a significant effect on rate of growth, diameter and crimp of wool fibres by changing the planes of nutrition.

An experiment, concluded at the Grootfontein School of Agriculture shows the influence of different planes of nutrition on two groups of Merino hamels in respect of body weights, fleece weights and fibre fineness. The sheep were fed on lucerne hay, mealies, oats and saltbush in varying quantities, these being common feeds used by the South African farmer.

2. PLAN OF THE EXPERIMENT.

A. Sheep and Management.

Twenty-four three-year-old Merino hamels were selected from the Grootfontein flocks that are bred and reared on Karroo veld. The sheep were subjected to a preliminary feeding period of four weeks. They were then divided into two groups of eleven each, two of the hamels being discarded as they reacted unfavourably to the rations.

Selection of the groups was based on body weights and a macroscopic examination of the fleece so that the groups were similar. The lots, recorded as 26 and 27, were then shorn, and shoulder samples of each sheep taken periodically for laboratory analysis. One animal of lot 27 died at the commencement of the experiment and one of lot 26 after nine months.

Each group was subjected to a different plane of feeding, 26 receiving a sub-maintenance ration, and 27 a full ration. Each lot was confined to a small enclosure 28 by 36 feet in which housing was provided with suitable feeding troughs and clean drinking water.

There were two distinct feeding periods. For the first nine months 26 received a sub-maintenance ration and 27 a full ration. For the second nine months half of lot 26 was placed on a full ration, and the remainder treated as before. Half of 27 was retained on a full ration and the remainder on a sub-maintenance ration. In this way for the second feeding period groups 26 and 27 each consisted of five well fed and five underfed sheep and served as a confirmatory test to the first nine months' period. The regrouping of lots 26 and 27 therefore resulted in:

5 sheep well-fed for 18 months;
5 sheep underfed for 18 months;
5 sheep well-fed for 9 months then underfed for 9 months;
5 sheep underfed for 9 months then well fed for 9 months.

Body weights were recorded weekly after the usual starvation period of fifteen hours. Shoulder wool samples were analysed periodically and the weights of fleeces recorded at shearing.

B. Feed.

The feed consisted of lucerne hay, crushed yellow mealies, whole oats and fresh oldman saltbush. To this ration was added a mixture of salt and bonemeal made in the proportion of 1:2. All feed was weighed before each feeding, which took place twice a day. The animals consumed all of their daily ration, an analysis of which is given in Table 1. The rations are given as averages for the periods mentioned and slight variations are not recorded.

TABLE 1.—AVERAGE DAILY RATION PER SHEEP.

Period.	Lot.	Treatment.	Ration in Ounces.					Nutrients in Pounds.			
			Lucorne.	Mealies.	Oats.	Saltbush.	Bonemeal and Salt.	Total Dry Matter.	Dig. Crude Protein.	Total Digestible.	Nutritive Ration.
Preliminary........	26 and 27	Well-fed	8	10	4	0	1	1·23	·13	·96	6·65
First 9 months.....	26	Underfed	5	5	3	6	1	·82	·09	·59	5·87
	27	Well-fed	8	10	4	8	1	1 35	·14	1·01	6·21
Second 9 months....	26C	Underfed	4	4	3	6	1	·71	·07	·51	5·78
	26D	Well-fed	8	10	4	8	1	1·35	·14	1·01	6·21
	27C	Underfed	4	4	3	6	1	·71	·07	·51	5·78
	27D	Well-fed	8	10	4	8	1	1·35	·14	1·01	6·21

3. EXPERIMENTAL RECORDS AND RESULTS.

A. Sheep Weights.

The live weights are summarized in Tables 2 and 3.

TABLE 2.

Average Live Weights (in lb.) for the First Nine Months.

Lot.	Treat-ment.	Initial.	Shorn.	1st 3 Months.	2nd 3 Months.	3rd 3 Months.	Per cent. Increase over 9 Months.
26	Underfed	90·7	83·0±2·21	82·2	79·5	75·5±2·00	− 9·4
27	Well-fed	92·1	85·1±2·51	89·2	95·0	101·9±2·65	+ 19·7

TABLE 3.

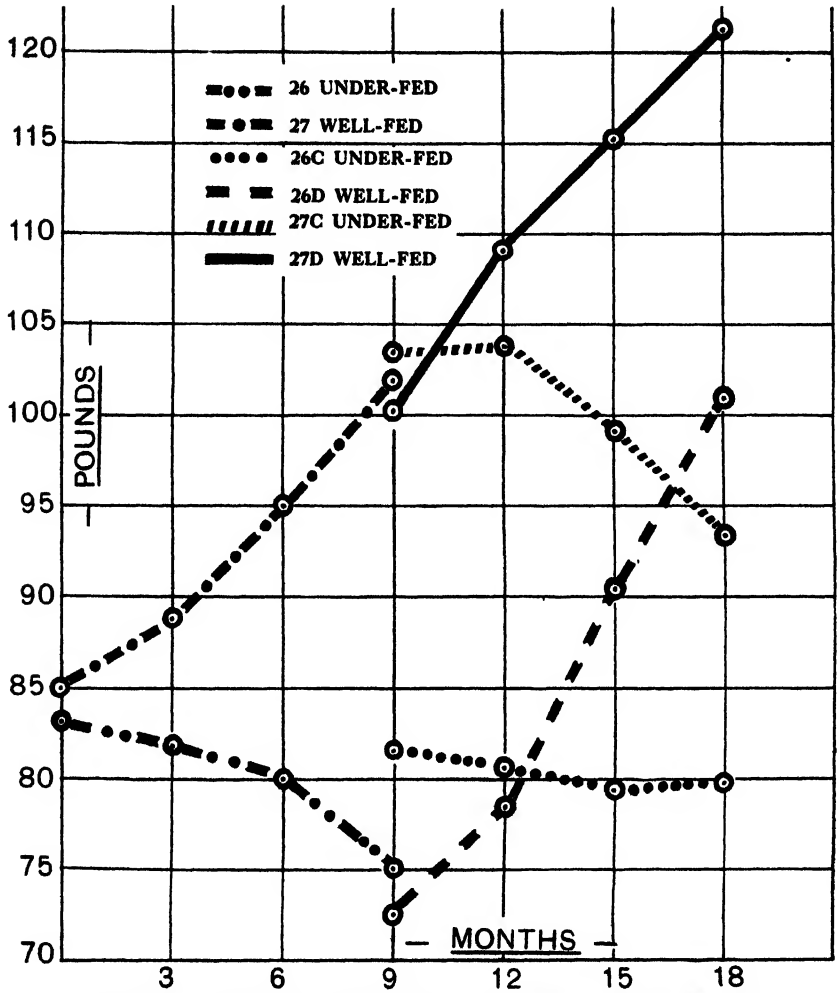
Average Live Weights (in lb.) for the Second Nine Months.

Lot.	Treat-ment.	Initial.	1st 3 Months.	2nd 3 Months.	3rd 3 Months.	Per cent. Increase over 2nd 9 Months
26C	Underfed	81·7±2·64	80·4	79·1	79·4±3·50	− 2·8
26D	Well-fed	72·6±1·70	78·5	90·4	101·0±3·90	+ 39·1
27C	Underfed	103·4±4·82	103·7	98·3	92·7±3·14	− 10·4
27D	Well-fed	100·4±2·11	109·1	115·4	121·2±4·70	+ 20·7

The initial shorn weights for the two groups after the pre-experimental period were 83·0±2·21 and 85·1±2·51 pounds respectively, which show no significant difference between lots 26 and 27 at the commencement of the experiment. The response of live weights to the different planes of feeding is illustrated graphically in Chart I. Photographs of the sheep are shown in Figures 1-4.

At the end of nine months, lot 26, the underfed group, gave an average of 75·5±2·00 pounds which shows a decrease of 9·4 per cent. on the initial weight. Lot 27, the well-fed animals, weighed 101·9 pounds or an increase of 19·7 per cent. The sheep were not shorn at the end of nine months, hence the weights include fleeces. In Table 2A are given the figures which take into account body weights only. The estimated body weight at the end of nine months was obtained by deducting half the grease weight of the fleece at the close of eighteen months from the live weight.

CHART I.



Showing the effect of feed on the live weights of the sheep.

TABLE 2A.

Average Body Weights for the First Nine Months.

Lot No.	Initial.	Estimated at End of 9 Months.	Percentage Increase.
26	83·0 ± 2·21	69·9 ± 1·89	− 15·8
27	85·1 ± 2·51	94·3 ± 2·52	+ 10·8

Fig. 1.—Showing the influence of feed on live sheep. Lot 26C. Underfed for 18 months. Av. body weight 68.9 pounds.

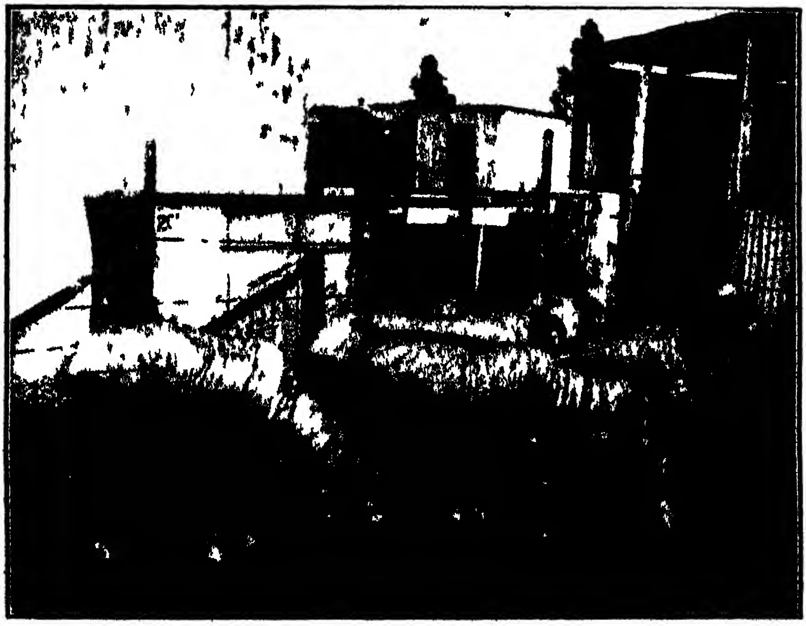


Fig. 2.—Showing the influence of feed on live sheep. Lot 26D. Underfed for 9 months. Well-fed for 9 months. Av. body weight 88.6 pounds.

The results differ slightly from those in Table 2 in that lot 26 now shows a bigger decrease, viz. 15·8 per cent. instead of 9·4 per cent. Lot 27 shows a smaller increase, viz. 10·8 per cent. instead of 19·7 per cent.

During the second nine months lot 26C was further reduced in weight to 79·4 pounds, a decrease of 2·8 per cent. Lot 26D gradually gained to 101·0 pounds or 29·1 per cent. when changed from the reduced ration to the full ration.

Lot 27C, that was formerly well-fed, decreased in weight to 92·7 pounds or by 10·4 per cent. when it was underfed. Lot 27D gained 20·7 per cent. when kept on the sam eration as for the first nine months.

The final live weights in Table 3 include fleece weights, while in Table 3A only body weights are taken into account.

TABLE 3A.

Average Body Weights for the Second Nine Months.

Lot.	Treatment.	Initial.	Final.	Percentage Increase.
26C...............	Underfed	76·5 ± 2·36	68·9 ± 3·10	− 9·9
26D...............	Well-fed	66·4 ± 1·70	88·6 ±. 3·53	+ 32·0
27C...............	Underfed	96·5 ± 4·65	78·8 ± 2·97	− 18·4
27D...............	Well-fed	92·0 ± 1·69	104·3 ± 3·22	+ 13·2

In Table 3A group 26C shows a decrease of 9·9 per cent. Group 26D which was changed to a full ration, showed an increase of 32 per cent.

Lot 27C was placed on a lower plane of feeding and decreased by 18·4 per cent. Lot 27D was kept on a full ration and increased by 13·2 per cent.

The percentage increase as reflected in Tables 3 and 3A differ slightly, but both serve to indicate the marked effect of feed on body weight.

B. Fleece Weights.

The sheep were shorn at the end of eighteen months and the wool produced compared on a scoured basis in Table 4.

TABLE 4.

Wool Production in Pounds.

Lot.	Treatment.	Grease Weight.	Scoured Weight.
26C....................	Underfed for 18 months	10·46	4·21
26D....................	Underfed for 9 months Well-fed for 9 months	12·36	4·65
27C....................	Well-fed for 9 months Underfed for 9 months	13·86	4·76
27D....................	Well-fed for 18 months	16·82	5·55

Fig. 3.—Showing the influence of feed on live sheep. Lot 27D. Well-fed for 18 months. Av. body weight 104.3 pounds.

Fig. 4.—Showing the influence of feed on live sheep. Lot 27C. Well-fed for 9 months. Underfed for 9 months. Av. body weight 78.8 pounds.

Lot 26C which was underfed for 18 months produced 4·21 pounds of scoured wool. Lot 27D was well-fed for 18 months and clipped 5·55 pounds which shows a difference of 1·34 pounds or 31·8 per cent.

Lots 26D and 26C, both starved for half the experimental period and well-fed for the other half produced 4·65 and 4·76 pounds respectively. These values do not differ significantly and are both an advance on lot 26C.

C. Fibre Fineness.

Wool sampling was confined to a closely clipped and marked shoulder area three inches in diameter. A staple for fibre thickness measurements was shorn every three months from each sheep so as to represent the growth for the three-monthly period. It was clipped in such a way that part of the place of sampling was common for consecutive shearings. Each three-monthly sample was cut into fragments, thoroughly mixed in ether and 250 fibres measured as a random selection on a Zeiss-Hegener Micro Camera.

TABLE 5.

Fibre Thickness in μ.

Groups.

Period.	26. Underfed, 11 Sheep.	26C. Underfed, 5 Sheep.	26D. Well-fed, 5 Sheep.	27. Well-fed, 10 Sheep.	27D. Well-fed, 5 Sheep.	27C. Underfed, 5 Sheep.
Initial.........	17·9 (80's)	—	—	17·7 (80's)	—	—
1st 3 months...	16·6 (90's)	—	—	17·2 (80's)	—	—
2nd 3 months..	14·9 (120's)	—	—	17·6 (80's)	—	—
3rd 3 months...	14·3 (150's)	—	—	16·9 (90's)	—	—
Initial.........	—	15·0 (120's)	13·6 (150's)	—	16·2 (90's)	17·7 (80's)
4th 3 months...	—	14·9 (120's)	16·4 (90's)	—	16·6 (90's)	15·9 (100's)
5th 3 months...	—	14·9 (120's)	17·5 (80's)	—	16·9 (90's)	13·7 (150's)
6th 3 months...	—	14·5 (150's)	17·8 (80's)	—	16·9 (90's)	13·4 (150's)

Note.—The equivalent spinning counts are given in brackets as determined by Duerden (1929).

The mean fibre thickness for the groups are compared in Table 5, and graphically in Chart II. The wool of group 26, the underfed sheep, became finer, ranging from 17·9μ, an 80's quality, to 14·3μ, a 150's quality. For the second nine months period group 26C, a sub-division of 26, remained fairly constant in fibre thickness when it received a ration similar to that of the first nine months. Lot 26D, the remainder of 26, were well-fed and recovered in fibre thickness to the initial quality number at the commencement of the experiment, namely an 80's.

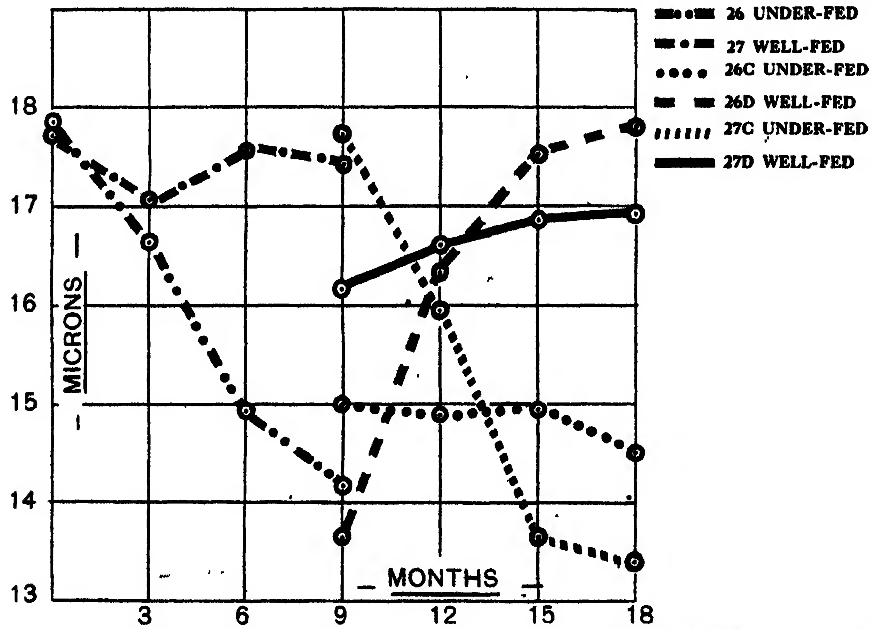
Lot 27, the well-fed animals, remained fairly constant for the first nine months, and so did its sub-division, lot 27D. Group 27C, the remainder of group 27, were underfed and the wool was refined to 13·4μ, a 150's quality. It is therefore shown that a wool can be changed from an 80's quality to a 150's quality by reduced feeding. The reverse, namely from a 150's to an 80's quality can be effected by changing the ration to a sufficient one.

The influence of feed on single wool fibres is demonstrated in photo micrographs in Figure 5. Fibre A, whe nit was well fed for the first nine months, showed a thickness of 35μ. During the second nine months, when it was underfed, the fibre thickness was reduced to 25μ, showing a thinning of 49 per cent.

Fibre B was underfed for the first nine months and then well-fed and showed an increase in thickness from 22·5μ to 27·5μ which is equivalent to 49 per cent.

Fibre C was well-fed for 18 months and remained constant in fibre thickness.

CHART II.



Showing the influence of the plane of nutrition on fibre thickness in microns.

DISCUSSION.

It is shown that quantity and quality in a Merino fleece largely depend on the nutrition of the animal. Underfeeding produces a smaller fleece although a finer fibred one. Good feeding produces a larger fleece but a coarser one. The market value of fine wool is higher than that of coarse wool. During droughts the sheep farmer will therefore expect a higher price per pound of wool but with a diminution of the quantity produced per sheep.

The marked influence of feed on the fleece is of importance where the hereditary characteristics of the Merino are studied. A control of the rations of the animals is necessary to place comparisons of the fleece attributes on a reliable basis when these are studied from one generation to the other.

The effect of the plane of nutrition on the Merino is of great significance to the Merino stud breeder. The characteristics of stud sheep can be changed to a marked degree by changing the feed, and this has often been the cause of disappointment to breeders when well-fed animals bought at high prices have been placed on the veld with less available food.

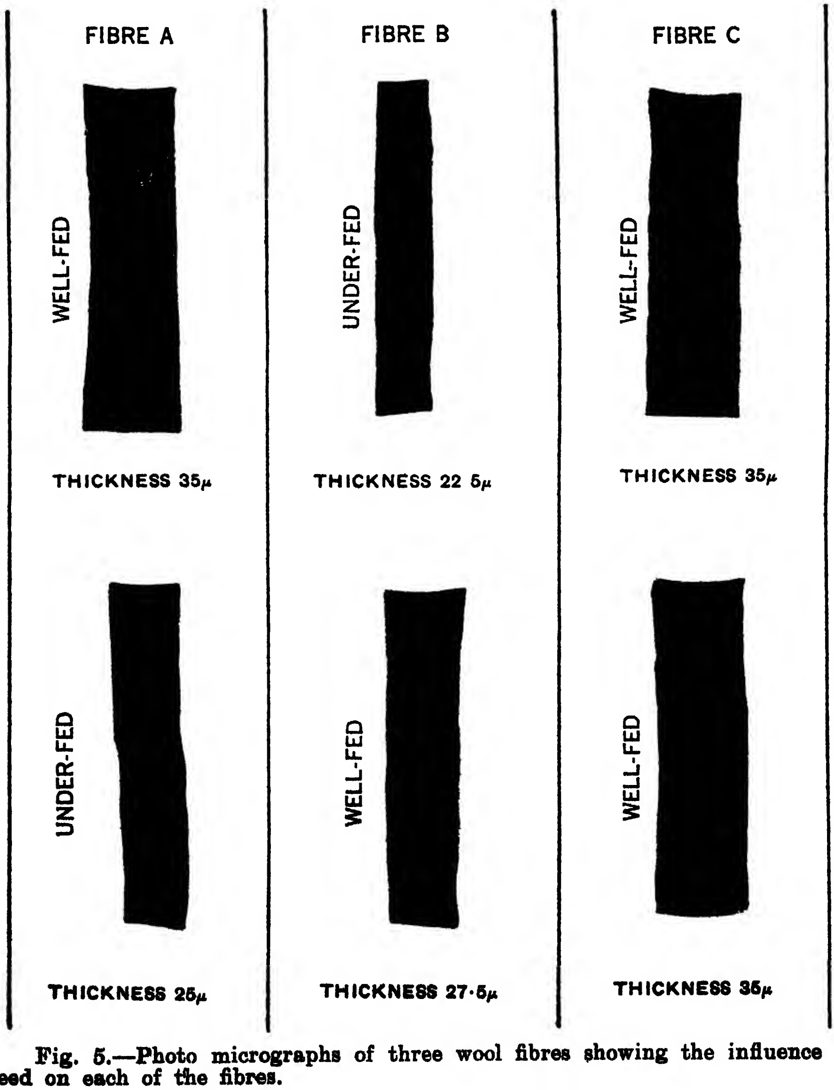


Fig. 5.—Photo micrographs of three wool fibres showing the influence of feed on each of the fibres.

In a study of Merino Stud Ram Wool (Bosman and Maré, 1933), the authors defined wool density as being dependent on the number of fibres per unit area as well as fibre thickness. Feed influences fibre thickness and therefore wool density. If it can be assumed that the number of follicles growing per unit area is not influenced by feed, then the diminution of wool density due to underfeeding is in proportion to the reduction in fibre thickness.

SUMMARY AND CONCLUSIONS.

The influence of feed on two groups of Merino hamels which were given different quantities of lucerne, mealies, oats, and saltbush was studied.

The body weights of the sheep were reduced by 15·8 per cent. when the feed was reduced. The sheep that were kept on a full ration gained 10·8 per cent. on their initial weights.

When the sheep were kept on a reduced ration for nine months and then placed on a full ration, their weights gained by 39·1 per cent.

Underfeeding reduced the scoured fleece weights by 31·8 per cent.

As regards fibre thickness the underfed group produced a considerably finer wool. The initial fibre fineness of 17·9μ was reduced to 14·3μ, a reduction of 36 per cent. in cross sectional area. The latter fibre thickness was restored to the initial stage by good feeding.

An 80's quality wool was reduced to a 150's by starvation and the latter restored to the original quality number by good feeding.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 3, Number 1, July, 1934.

Further Notes on Lumpy Wool in South Africa.

By

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BULL (1929) reported the isolation of a micro-organism, *Actinomyces dermatonomus*, from the natural lesions of a skin disease of sheep designated by him as *Actinomycotic dermatitis* and popularly referred to as "Lumpy wool". Bull described the morphological, cultural and pathogenic characteristics of this organism and assigned to it the causative rôle in the production of the condition. Albiston (1933) described the natural occurrence of this disease in calves. He was able to isolate an organism similar to that described by Bull and to transmit the condition to sheep and calves.

THE OCCURRENCE OF THE DISEASE IN SOUTH AFRICA.

In some localities, e.g. in the Humansdorp district, the disease assumes serious proportions during certain seasons. However, in most other areas only one or two cases occur in the flock and under these conditions there is no evidence to show that the disease is very contagious. There appears to be no doubt that rainfall and humidity are very important in connection with the occurrence of this disease. The following points with regard to the incidence of the disease in South Africa illustrate this:—

1. Hitherto only isolated outbreaks have been noted in the Karroo districts. This tract of country is an important sheep-raising area and is arid or semi-arid.

2. Most of the outbreaks which have been reported have occurred in East Griqualand and the eastern, south and south-western districts of the Cape. The rainfall in these areas is relatively high.

3. In the Humansdorp district (southern Cape) a large number of cases occurred in 1932 when the district experienced abnormally heavy rains. However, in 1933 when much less rain fell and droughty conditions prevailed, no fresh cases were reported and most of the sheep which developed the disease in 1932 recovered spontaneously after shearing.

TYPE OF ANIMALS AFFECTED.

As far as it has been possible to ascertain the disease has been noted in Merino sheep only. However, the fact that Merinos preponderate in South Africa may account for the apparent non-susceptibility of other breeds. It would appear that lambs are particularly susceptible, although cases in older animals are not uncommon.

THE ISOLATION OF THE CAUSATIVE ORGANISM.

It was possible to isolate the organism, *Actinomyces dermatonomus*, from two affected sheep (D.O.B. Nos. 32922, 33501). These sheep were sent to the Onderstepoort laboratory from two widely separated districts of the Union.

In these animals the presence of the organism, in the form of mycelia, could readily be demonstrated in smears prepared from the diseased skin.

To obtain material for culture a lump of matted wool was gently peeled away from the skin and from the exposed raw surface some exudate was sown on serum agar, and this incubated at 37° C.

The cultural and morphological characteristics of the organism are like those described by Bull.

MORPHOLOGY.

Depending upon the age of the culture the morphology varies greatly. The growth on blood or serum agar after 24 hours incubation consists of branching mycelia with an occasional condium. The mycelia vary greatly in size, being usually 2-6 μ long and 0·5-1·0 μ thick.

Frequently protruberances are present in the course of a filament and very often the mycelia terminate in knob-like enlargements. After 48 to 72 hours the mycelia are less numerous and appear to be broken up. The culture now consists chiefly of conidia. At this stage mycelia are found which terminate in a series of coccus-like bodies. After a further 4-6 days incubation the smears from the culture resemble those of a pure culture of a small coccus.

The organism stains readily with the usual aniline dyes and is Gram-positive.

CULTURAL CHARACTERISTICS.

The organism grows easily and well on the usual laboratory media. The addition of serum or blood leads to a more luxuriant growth. At 37° C. optimum growth is obtained, but even at ordinary room temperature (20-26° C.) growth occurs, and after 4 or 5 days satisfactory cultures are obtained. The organism is strictly aerobic. On serum- or blood-agar and at 37° C. a dirty white, somewhat raised and rough growth is obtained after 24 hours. The growth increases with further incubation and becomes wrinkled or corrugated and slimy. Individual colonies are roughly round, with an undulating but entire border and the surface is wrinkled. The colour varies from dull white, grey to yellow. The surface is occasionally mucoid in consistency. Colonies aged 48 hours to 4 days are buried in the medium and can be removed *in toto* only with difficulty. On blood agar (particularly in poured plates) definite although not marked haemolysis is present around each colony.

On 1 per cent. glucose agar the appearance is similar to that on serum agar, but the growth is less profuse. In beef infusion peptone broth good growth occurs in 24 hours in the form of small floccules. After 4 days a flocculo-stringy deposit is obtained. A similar, though not so profuse growth is obtained in 1 per cent. glucose broth. In 5 per cent. serum (sheep) broth better results are obtained; a heavy

flocculent turbidity with, later, a slight scum-like pellicle forms. The organism grows very poorly in meat broth, i.e. broth plus meat particles.

Inspissated serum (horse) medium becomes softened and semi-solid.

Litmus milk is completely digested after 7-14 days incubation.

Gelatin is liquefied.

Fermentation Reactions.

(Note.—The various carbohydrates were added to make up 1 per cent. solutions in 1 per cent. peptone water. The inoculated tubes were incubated for 14 days at 37° C. before readings were taken). It was found that acid but no gas was produced in glucose, laevulose, maltose and dextrine. No fermentation was observed in galactose, salicin, lactose, mannite, adonite, xylose, sorbite, inulin, raffinose and in inosite.

Fig. 1.—An advanced case of dermatomycosis; note lesions around the mouth and on shoulder region.

In Clark and Lubs phosphate medium a doubtfully positive methyl-red reaction was obtained; the Voges-Proskauer reaction was negative. The production of neither indol nor ammonia could be demonstrated in peptone water. Nitrates were not reduced to nitrites.

Pathogenicity.

(*a*) *Laboratory animals.*—The results are in entire agreement with those of Bull.

The application of either culture or ground up material from natural cases to the skin produces in the rabbit, guinea pig and mouse a lesion resembling that got in sheep.

In the guinea pig and mouse the lesion is mild; after 24 hours a reddening of the scarified area appears, followed by the formation of a superficial crust. The crusts peel off after 4 or 5 days leaving a completely healed skin.

The rabbit reacts in a more satisfactory manner, particularly, as Bull pointed out, if the hair is plucked out. The crust formation may be of considerable extent and thickness, but when this peels off the underlying skin is completely healed as in the case of the guinea pig and the mouse.

The application of emulsified material from natural lesions to the scarified or plucked skin of the rabbit has proved to be a suitable method for demonstrating the presence of the organism. From the lesions, practically pure cultures of the organism may be obtained.

(*b*) *Sheep.*—It was possible to set up reactions in sheep in the following ways:—

(1) With culture material applied to the intact skin and to slightly scarified areas;

(2) with emulsified lumps from natural cases applied to lightly scarified areas.

Lesions on the intact skin could be provoked only by using a large amount of culture and keeping the wool moist for a few days.

The development of the lesions are most easily followed on woolless portions of the skin, e.g. inside the thighs. On such sites a distinct hyperaemia of the scarified area is first noted, this being accompanied by a thickening of the skin. Soon small papules (filled with yellow material) appear. These enlarge and coalesce, and later rupture, the purulent material drying to form scabs. In experimental cases the lesions are usually of a transitory nature, this being especially the case on woolless areas. After about 3 weeks, healing commences and the scabs, which are now about $\frac{1}{8}$ inch to $\frac{1}{4}$ inch thick, peel off. The underlying skin heals completely and no cicatrices are formed. In one sheep where a large amount of broth culture was applied to a portion of intact skin along the side of the body, and which was covered with about 6 months' growth of wool, a chronic lesion developed. After three months this lesion was still active and in all respects resembled that of a natural case.

The Probable Method of Infection under Natural Conditions.

As has been mentioned, outbreaks of the disease occur sporadically, and in South Africa the condition can definitely be associated with the rainfall. It is possible that Bull's theory of the normal saphrophytic nature of the organism is correct, and that it becomes pathogenic and invades the skin when the latter is in some or other way injured, as, for example, after prolonged wetting during a very rainy spell of weather.

In view of this an attempt was made to isolate the organism from the skin and wool of 12 normal lambs, aged about 12 months. The skin of each sheep was scraped in three different sites with a blunt knife, the scrapings shaken up in a small quantity of saline, and poured serum agar plates prepared from this material. The plates

were incubated at 37° C. and examined daily for four days. Colonies in any way resembling those of *Actinomyces dermatonomus* were picked and replated. It was impossible to demonstrate the presence of the organism on the wool or skin of these animals.

Methods of Treatment and Prevention.

Steyn (1931) has advised treatment with a mixture of tincture of iodine and linseed oil. Good results were obtained with this in those cases where the lesions were not very extensive and of fairly recent origin. Where, however, very extensive lesions were present the application of this mixture did not yield the desired results. From the point of view of economy it would hardly seem justified to treat by this method, ordinary flock sheep which have such extensive lesions; indeed, Steyn advises that such animals should be destroyed. Where the lesions are well developed and firmly adherent to the skin, it is difficult to conceive of a fungicidal preparation having sufficient penetrative properties to effect a cure. Such cases must be treated surgically and the hard lumps carefully removed and the exposed skin treated with a suitable fungicidal preparation, such as a solution of copper sulphate. Previous softening of the crusts with any oily preparation can be recommended.

The general experience of farmers is that when infected sheep are shorn most cases recover without any special treatment. During the shearing operations the hard lumps are usually removed and the lesions which are found mostly on the dorsal regions of the body, become exposed to the sunlight. Apparently this is quite sufficient to bring about sterilisation of the wound and ultimate healing.

An experiment was planned in collaboration with Government Veterinary Officer E. T. Clemow, with a very badly infected flock in the Humansdorp district to ascertain the value of certain dips for preventing and curing the condition. The infected sheep were marked at shearing time. Infected and clean sheep were dipped in lime and sulphur and in Cooper's Double Dipping powder. Suitable control groups were retained. The results are summarised in the following table:—

Group No.	October, 1932, No. of Sheep in Group.	Dipped in.	Result, i.e. Percentage Found Infected, October, 1933.
A_1 (clean)................	100	Cooper's Double Dipping Arsenical Powder	9·0
A_2 (infected).............	200	,, ,, ,,	8·5
B_1 (clean)................	653	Lime and sulphur (Capex)	4·0
B_2 (infected).............	354	,, ,, ,,	3·4
C_1 (clean)................	100	Not dipped (controls).....	4·0
C_2 (clean)................	100	,, ,, ...	4·0

It will be noticed that:—

(1) During the season 1932, 654 sheep out of a total of 1,507 in this flock, or about 43 per cent., showed infection. The lesions were of varying extent and degree.

(2) The dipping of clean and infected sheep in two commonly used dipping preparations had very little effect on curing or preventing the disease.

(3) The incidence of infection during the season 1933 was 43 per cent. and during 1932, 6 per cent. During these two seasons the rainfall was 40·85 inches and 25·54 inches respectively, clearly indicating that the occurrence of the condition is associated with conditions of moisture.

A solution of copper sulphate, as advised by Bull, for preventing this condition, was not tried. It should be remembered that when sheep are dipped in an aqueous solution of copper sulphate there are materials such as carbonate, etc., in the suint, which will form insoluble copper compounds, with the result that the first few animals dipped tend to carry down the greater proportion of the copper and the dipping solution will then have less fungicidal effect.

Summary.

(1) The occurrence of further outbreaks of lumpy wool in South Africa is reported.

(2) The isolation and morphological and cultural characters of *Actinomyces dermatonomus* are described.

(3) The production of the lesions of lumpy wool in laboratory animals and in sheep by the use of cultures and material from naturally infected sheep is recorded.

(4) The probable method of natural infection, and curative measures are discussed.

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The Determination of Fleece Density in the Merino Sheep.

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INTRODUCTION.

THE term " density " is used by the practical sheep judge to denote compactness in a fleece. In discussing the " Interpretation of the Merino Score Card in South Africa ", Rose (1930) refers to density as meaning " the close proximity of fibre growth on a given surface of skin ".

Several research workers have attempted a more precise definition. Duerden and Botha (1930) suggest either " the volume of wool material of a certain length " or " the weight of wool material of a certain length, grown on a constant skin area ".

Burns and Miller (1931) in their work on " Sampling Instruments to Determine Fleece Density in Sheep ", refer to density as meaning " the total amount of wool fibre growing on a definite unit area of skin, usually on the living animal ".

Workers are agreed that the expression for fleece density is based on the number of fibres growing per unit area of skin. Some use this factor only. (Hultz and Paschal, 1930; Burns, 1933). Others suggest that number of fibres per unit area should be associated with fibre fineness. (Bosman and Maré, 1933; Bosman, 1934.)

Whichever system for expression is used, the number of fibres growing per unit area is the basis and its estimation is necessary in fleece analysis. The determination of fibre fineness has received the attention of many workers (Duerden, 1929; Roberts, 1927; Barker and Burgess, 1928, *et alia*).

The study outlines two methods for determining the number of fibres growing per unit area of skin and compares their relative merits for routine anlysis of merino fleeces in sheep breeding.

METHODS.

A. SAMPLING.

Burns and Miller (1931) review the sampling instruments used to delimit unit area of skin on the sheep. Some of these were tested by the author on the dense merino fleece, and of these the Wyedena Fleece Caliper (Burns and Miller, 1931) was found useful and was adopted. The caliper delimits four square centimetres of skin area and the wool is cut with a fine nail scissors from the area. Repeat tests on the same region of the animal have shown great consistency.

In our genetic studies sampling is made on different regions of the sheep in order to measure variability in density. A similar system of regional sampling was used by Burns (1933).

B. Estimating the Number of Fibres.

To estimate the number of fibres in the sample, most workers have adopted the method of counting a portion of the fibres, and then by comparing the weight of the portion with that of the large sample, form an estimate of the number. (Burns, 1931; Duerden and Botha, 1930; Bosman and Maré, 1933.)

In the present study an account of the weight-volume method is given and a comparison is made between results obtained by it and those by the counting method.

A series of samples obtained by means of the Wyedena Fleece Caliper on Merino Stud Ewes was analysed by both the counting method and the weight-volume method. Each sample was cleansed from wool grease and other impurities by the benzene-saponin method described by Miller and Bryant (1932). During scouring care was taken to keep the fibres undisturbed in the sample so as to avoid any matting. Each sample was handled by means of forceps and after drying, five hundred fibres were counted from the skin end of the staple. The bundle of counted fibres and the large sample were allowed to stand in the balance case before weighing so that both were weighed under similar conditions. By simple proportion the number of fibres was then calculated.

The Weight-Volume Method.

The alternate method by weight-volume was used on the same samples. This method was also suggested by Burns and Miller (1931) and takes into account dry weight of the sample, the volume of wool (from a knowledge of the length of the fibres and their fineness) and the specific gravity of wool. The number of fibres is calculated by the formula:—

$$N = \frac{W}{S \times A \times L}$$

where N is the number of fibres, S the specific gravity of wool adopted as a constant 1·30 (King, 1926), A the mean cross-sectional area of the fibres, and L, their mean straight length.

The dry weight of wool was obtained by the method of Barrit and King (1926). A current of dry air maintained at 105° C. was passed through the wool sample which was contained in a specially designed Regain Bottle. The cross-sectional area of the fibres was calculated from the mean fibre fineness measured by the Micro-Camera method described by Duerden (1929).

The straight length was obtained by measuring 50 fibres at random from each sample according to the method of Burns (1931).

Experimental Results.

The two methods were tested on twenty-six Merino stud ewes and the results summarised in table form. A close agreement between the two is shown. The number of fibres growing per square centimetre and per square inch are given in each case. In the last two columns of the table is given the mean fibre fineness and the fleece density in terms of per cent. skin area occupied by wool fibres.

TABLE 1.—COMPARISON OF RESULTS OBTAINED BY TWO METHODS.

Sample.	By the Method of Counting 500 Fibres.		By the Weight-volume Method.		Mean Fibre Thickness in μ.	Fleece Density as Per Cent Skin Area.
	No. of Fibres per Sq. Cm.	No. of Fibres per Sq. Inch.	No. of Fibres per Sq. Cm.	No. of Fibres per Sq. Inch.		
1	6,500	41,930	6,478	41,790	19·09	1·85
3	9,295	59,970	9,257	59,730	19·05	2·64
4	8,615	55,580	8,607	55,520	19·64	2·61
5	8,990	58,000	8,985	57,970	20·60	2·99
6	8,292	53,500	8,115	52,350	17·46	1·94
7	6,905	44,550	6,992	45,110	20·60	2·33
8	6,110	39,420	6,087	39,270	20·37	1·98
9	6,430	41,480	6,470	41,740	19·29	1·89
10	9,000	58,060	9,150	59,030	20·76	3·10
11	8,327	53,730	8,300	53,550	20·62	2·77
12	5,760	37,160	5,930	38,260	21·48	2·15
13	6,450	41,610	6,465	41,710	18·95	1·82
14	7,435	47,970	7,392	47,690	20·73	2·49
15	6,025	38,730	6,032	38,920	21·67	2·22
16	5,552	35,820	5,550	35,840	20·91	1·91
17	5,750	37,100	5,815	37,516	22·30	2·27
19	7,362	47,500	7,315	47,190	20·26	2·36
20	6,850	44,190	6,820	44,000	17·09	1·56
22	10,607	68,440	10,530	67,940	16·93	2·37
23	6,340	40,900	6,457	41,660	21·85	2·42
24	7,015	45,260	7,107	45,850	20·73	2·40
25	9,370	60,450	9,300	60,000	20·62	3·10
26	5,652	36,470	5,637	36,370	19·27	1·64
27	5,907	38,110	5,932	38,270	21·25	2·10
29	7,000	45,160	7,070	45,610	18·03	1·80
30	6,070	39,160	6,100	39,350	22·57	2·44

DISCUSSION.

The weight-volume method for estimating number of fibres per unit area gives results that agree closely with those obtained by the counting-weighing method. The author found the former preferable to the latter and has adopted it for routine analysis of density determinations in merino genetic studies. The weight-volume method has its advantages in the fact that it eliminates the laborious counting of 500 fibres from each sample. The factors necessary in its calculation are dry weight of the sample, straight fibre length and mean fibre thickness. The latter two are determined in the ordinary course of fleece analysis in genetic studies and the only determination necessary is the dry weight.

Separate complete tests have shown that the weight-volume method is quicker than the 500 counting method when a large number of routine samples is analysed. With a convenient heating apparatus that holds a number of Regain Bottles, the determination is greatly facilitated.

The number of fibres per square centimetre as well as per square inch is given, as both systems have been employed by research workers.

The fibre fineness and the fleece density expressed as per cent. skin area occupied by wool fibres are given. As was shown by the author (1934), the number of fibres alone does not express fleece density. For example in the table, Sample No. 22 with 10,530 fibres per square centimetre and a mean fibre thickness of $16 \cdot 93\mu$ is less dense with 2·37 per cent. fleece density than Sample No. 25 which has 9,300 fibres per square centimetre, a fibre thickness of $20 \cdot 62\mu$ and a fleece density of 3·10 per cent.

Density determinations are becoming increasingly important in fleece analysis since density plays a very significant rôle in wool production. In a study of Rambouillet Show Sheep, Hultz and Paschal (1930) attached primary importance to fleece density when sheep judges' awards were analysed.

In an analysis of fleece characteristics as they affect wool production the author (1933; 1934) shows that density is of considerable importance. On a merino stud ram that has twelve square feet skin area, a four-inch staple length, and a 64's quality wool, every ten thousand fibres per square inch add 2·36 pounds to the scoured fleece weight. Such a ram with fifty thousand fibres per square inch would produce 11·8 pounds of scoured fleece.

SUMMARY.

An analysis is given of fleece density by two methods, the counting-weighing method and the weight-volume method and a close agreement between the results is shown.

Preference is given to the weight-volume method when in the course of genetic studies on merino sheep a number of routine samples have to be analysed.

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The Fibre Fineness of South African Merino Wool.

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INTRODUCTION.

Merino Wool is characterized by fibre fineness. In a survey of the world's wool, published by the Empire Marketing Board (1932), the report differentiates three main types: Merino, Crossbred and Carpet, the basic distinction depending on fibre fineness. It was also shown that Merino wool has the largest range of quality numbers with limits from 60's to 100's, that Crossbreds range from 36's to 58's and Carpet from 22's to 34's.

Several workers have used fibre fineness for classifying the different quality numbers of Merino wool. (Duerden, 1929; Winson, 1931; Plail, 1930; Dantzer and Roehrich, 1931; Barker and Winson, 1931, *et alia*).

Different authors have used different systems for expressing fibre fineness. Some have adopted fibre thickness or fibre width, others have used the weight-length method.

Fibre fineness and quality numbers of South African Merino wool form the subject of the present study. The method of measurement and the standards for classification are those established by Duerden (1929), who included the range from 60's to 100's as well as a 120's and a 150's, since some Merino wools are finer than 100's quality number.

The micro-camera projection method for measuring fineness which has been adopted has definite advantages over other methods such as the Gravimetric (Roberts, 1927) or the Diffraction Method (Duerden, 1929), in that it demonstrates the fibre variability within the sample, an important wool factor (Barker, 1931) and is a quick and ready method for routine analysis. In addition to fibre fineness an analysis is given of fibre variability, crimping and the coefficients of correlation.

MATERIAL.

South African Merino wool is produced under very varying conditions, and a representative selection needs to be based on a large number of samples so that this is taken into account. In the present study the collection consists of one thousand wool samples obtained from woolmen at the coastal ports and from South African wool farmers. The material is in the form of small and large wool samples and many represent an average selection from bales and clips. There are included wools grown under droughty conditions and in seasons of plenty; Karroo grown and Grassveld grown, and wools from all the four provinces of the Union of South Africa.

In a recent publication (Bosman and Botha, 1933), the authors describe an analysis of stud ram wool from leading Merino stud breeders in South Africa. It was shown that ram wool requires different standards of classification from the general commercial clips. The proportion of wool of this type in the Union's wool has been estimated by sheep officers as one to two per cent., and this is baled separately according to the standards of the National Wool Grower's Association. Ram wool is not included in the present study which only takes into account the general South African commercial clips.

METHODS.

Individual samples were analysed for fibre fineness by the Camera method (Duerden, 1929). Fibre fineness is expressed as the mean fibre thickness or fibre width.

In order to obtain a representative mean thickness of the sample, a system of random sampling was used. This takes into account the variability of wool.

Each sample was divided into smaller lots and a random selection of staples taken from each, so that ultimately these consisted of 100 to 500 staples from the original sample, the number depending on the size of the sample. A wool strand was taken from each staple and all the strands were rolled together. These were further cut into small clippings at about eight to ten places along the staple length so as to average up any variability along the length of the staple (Duerden and Bosman, 1927).

The small clippings were mixed in ether until a mixture of uniform dispersion was obtained. The fibres were dried and mounted in Euparal for the measurement of 250 on a Zeiss Hegener Micro camera. As was shown by Duerden (1929), test measurements of different slides of the same sample prepared in the above manner revealed great consistency and the method is a reliable one for obtaining mean fibre thickness.

Experiments have also shown that the mean fibre fineness expressed as fibre width of a large number of fibres does not differ materially from results obtained by using the cross-sectional area method, or the gravimetric method.

The determination of fibre fineness by the Camera method has given the frequencies of fibre thickness. It was thus possible to obtain the standard deviation and the coefficient of variability of each of the thousand samples.

EXPERIMENTAL RESULTS.

1. Fibre Thickness and Distribution.

One quarter of a million fibres were measured for the study which is based on a random selection of South African Merino wool. The grouping of the fibre measurements according to class intervals of $2 \cdot 5\mu$ is shown in Table 1, where the frequencies and percentage frequencies are given in the second and third columns.

Table 1.—Fibre Distribution of South African Merino Wool.

Fibre Thickness in μ.	Frequency.	Per cent. Frequency.	
7·5	330	·13	
10·0	5,013	·20	
12·5	23,869	9·48	
15·0	40,737	16·18	83 per cent.
17·5	62,082	24·66	
20·0	51,882	20·61	
22·5	29,325	11·65	
25·0	19,524	7·76	
27·5	8,350	3·32	
30·0	4,943	1·96	
32·5	2,963	1·18	
35·0	1,228	·49	
37·5	853	·34	16 per cent.
40·0	245	·10	
42·5	178	·07	
45·0	84	·03	
47·5	82	·03	
50·0	62	·02	
	Total 251,750		

It is of interest to note that the finest fibres measure 7·5μ and the coarsest 50μ. Eighty-three per cent. of the fibres range from 7·5μ to 22·5μ and the remaining sixteen per cent. have their limits between 25μ and 50μ. The latter limits are coarser than a 60's quality number and not strictly classified as Merino.

The mean fibre thickness of all the fibres is 19·11μ or a 66's quality number. The standard deviation is 4·868μ and the coefficient of variability 25·5 per cent. The frequencies of the table are represented graphically in Figure 1.

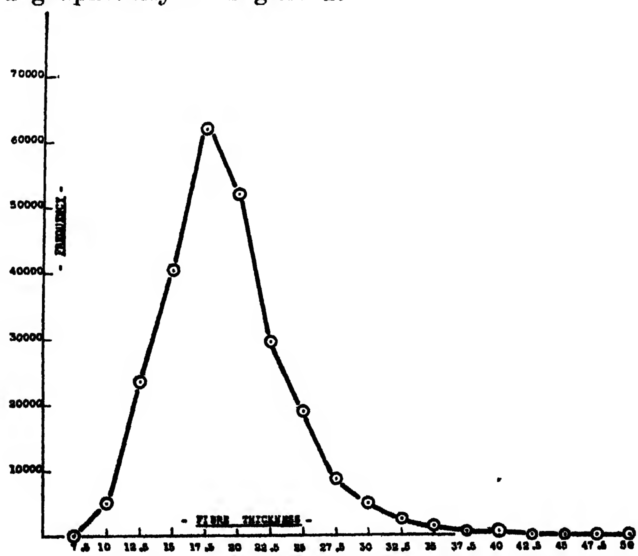

Fig. 1. Frequency distribution of a quarter million South African Merino wool fibres arranged in classes according to fibre thickness.

The mode at $17 \cdot 5\mu$ shows a frequency of 24·6 per cent.

QUALITY NUMBERS OF INDIVIDUAL SAMPLES.

The frequencies and percentage frequencies of the quality numbers are summarized in Table 2.

TABLE 2.—QUALITY NUMBERS OF SOUTH AFRICAN MERINO WOOL.

Quality Number.	Frequency.	Per cent. Frequency.	
150's	57	5·7	92 per cent.
120's	58	5·7	
100's	49	4·9	
90's	96	9·5	
80's	133	13·2	
70's	166	16·5	
66's	159	15·8	
64's	127	12·6	
60's	79	7·8	
58's	50	5·0	8 per cent.
56's	26	2·6	
54's	7	0·7	
Total	1,007		

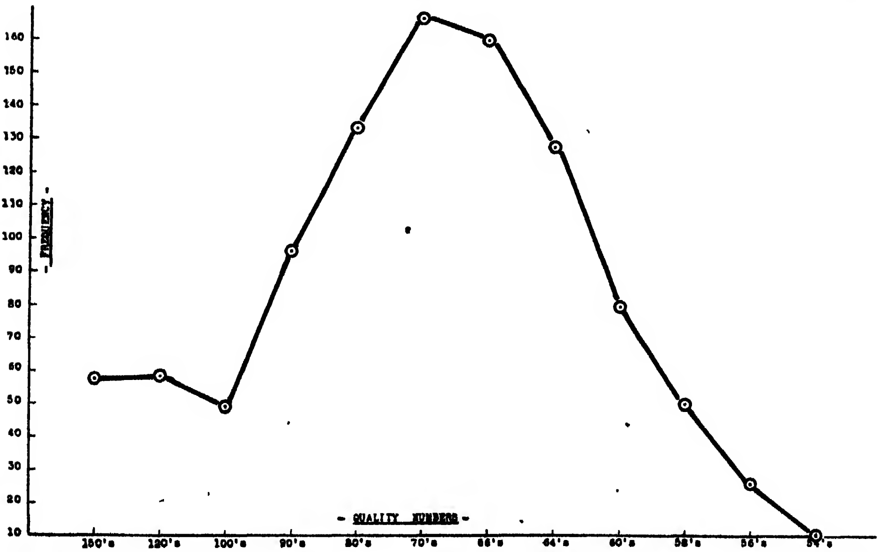

Fig. 2.—Frequency distribution of one thousand Merino wool samples arranged in classes according to quality numbers.

The percentage frequencies show that 92 per cent. of the samples fall strictly within Merino quality numbers. Eight per cent. is coarser and is classed as cross-bred. It may be noted that 25·8 per cent. range from 90's to 150's. These include wools grown under droughty conditions and a portion of the samples are tender. Many have sufficient tensile strength to be passed as "sound". It is, therefore, demonstrated that the South African Merino clip includes a small proportion of superfine wools that conform to a fineness of 90's to 150's.

When classification is based on the fineness types adopted by the National Wool Growers' Association and classed as "Strong", "Medium", and "Fine" Merino then approximately eight per cent. are strong of 60's quality number, twenty-eight per cent. are medium with a 64's to 66's, and fifty-five per cent. are fine, and consist of 70's quality number and finer. Eight per cent. are cross-bred.

The frequencies in Table 2 are shown graphically in Figure 2.

Variability.

Uniformity is an important wool attribute to the textile manufacturer. Several authors have made reference to its value. (Roberts, 1930; Barker, 1929, *et alia.*) Barker (1931) asserts that "it is not only the average fineness of a sample and its frequency distribution, but also its coefficient of variation within the sample, that is of supreme importance".

The fibre variability of the South African Merino samples, expressed as coefficient of variability, is summarized in Table 3. The coefficient of variability arranged in class intervals that differ by two per cent. is given in the first column of the table. The frequency and percentage frequency are shown in the second and third columns.

Table 3.—Coefficient of Variability.

Coefficient of Variability (per cent.)	Frequency.	Per cent. Frequency.	
10–12	5	·5	10·8
12–14	20	2·0	
14–16	84	8·3	
16–18	179	17·8	79·0
18–20	289	28·7	
20–22	195	19·4	
22–24	132	13·1	
24–26	70	6·9	10 4
26–28	21	2·1	
28–30	7	·7	
30–32	7	·7	
Total	1,007		

As regards coefficient of variability it is shown that the samples have a range from 10 to 32 per cent. Approximately 79 per cent. range from sixteen to twenty-four per cent. coefficient of variability. Eleven per cent. are more uniform with ten to sixteen per cent. coefficient of variability. Ten per cent. are more variable with values from 24 to 32 per cent. coefficient of variability.

The frequencies of the coefficients of variability are given graphically in Figure 3.

Correlations: Fibre Fineness and Crimps.

In wool buying and selling practice, crimping is often used as an aid to estimate fibre fineness or quality number. It is held that fibre fineness is associated with size of crimps or number of crimps per inch.

Several workers have investigated the relationship between fibre fineness and crimping.

Davenport and Ritzman (1926) found no significant correlation between the two factors.

Duerden (1929), showed an agreement between the two characteristics when the wools were not impoverished and not associated with skin folds. On well grown wools definite standards for crimping and fibre fineness were established.

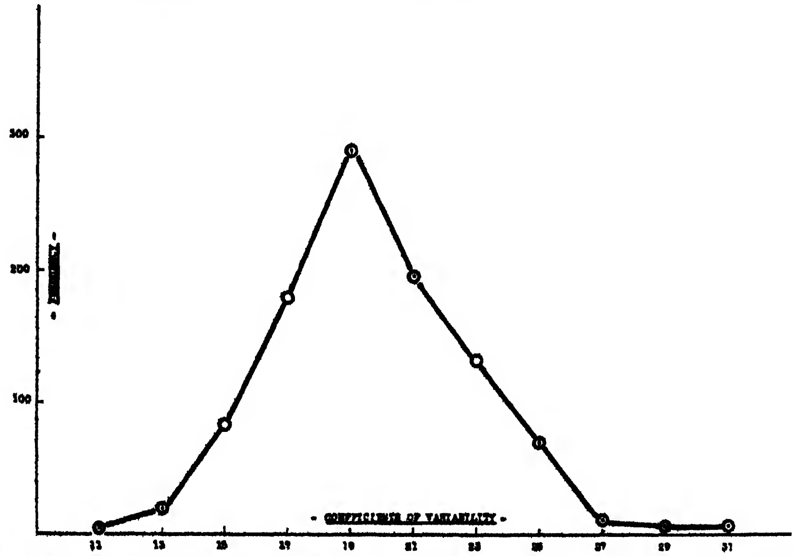

Fig. 3.—Frequency distribution of one thousand Merino wool samples arranged in classes according to co-efficients of variability.

Reimers and Swart (1929; 1931), showed that no definite relationship between average fibre diameter and crimping existed.

Hultz and Paschal (1930) found an insignificant negative correlation between crimp and fibre diameter in wool studies on Rambouillet sheep.

Bosman and Botha (1933) in their work on shoulder samples from Merino stud rams found a negative correlation coefficient between crimping and fibre fineness which was definite though not high.

In the present study the relationship of the characteristics of fibre fineness and crimping are compared. The study is based on samples that include droughty and well grown wools, those from skin folds and on different regions of the body of the sheep. Ram wool is, however, excluded as this type is usually baled separately from the general clips.

The coefficient of correlation between crimps and fibre fineness on 340 samples selected at random from the 1,000 available for study, is given as $-\cdot617 \pm \cdot0226$. This indicates a definite correlation, although not a perfect one. In general fine fibred wools have more crimps per inch than coarse fibred ones.

Although a very general relationship between the two characteristics exists the number of perfect agreements obtained on individual determinations is not high. In the study it is shown that:—

121 samples or 36 per cent. are coarser in fineness than the crimps indicate.

93 samples or 28 per cent. show absolute agreement in both standards.

120 samples or 36 per cent. are finer in fibre thickness than the crimps indicate.

Of the 121 samples that were coarser in fibre thickness than the crimps showed, 60 were coarser by one quality; 39 by two qualities and 22 by more than two qualities.

Of the 120 samples that showed a finer fibre thickness than the crimps indicated, 56 were finer by one quality, 43 by two qualities and 21 by more than two quality numbers.

Fibre Fineness and Coefficient of Variability.

The coefficient of correlation between fibre fineness and coefficient of variability was $-\cdot0754 \pm \cdot0217$ which shows no significant correlation between the two factors. When coefficient of variability is taken as the measure of uniformity then fine wools have the same degree of variability as coarse wools. This result confirms that obtained by Bosman and Botha on Stud Ram Wool (1933).

DISCUSSION.

The study demonstrates fundamental characteristics of South African Merino wool, which as a raw material forms the major pastoral product of the Union.

Eighty per cent. of South African Merino wool fibres measure from $12\cdot5\mu$ to $22\cdot5\mu$ and this fineness can be regarded as characteristic of the bulk of the South African wool clip.

As regards quality number, eighty per cent. of the samples conform to the Merino quality numbers of 60's to 100's. Eleven per cent. are finer than 100's and are classed as 120's to 150's. The superfine wools are presumed to be the result of droughts and are produced under adverse conditions of feeding. Experimentally it has been shown that lack of nutrition produces this extreme fineness (Maré and Bosman, 1934). Eight per cent. are cross-breds and are coarser than Merino wool.

In the study, fibre fineness is regarded as the basis for classification into the fineness types. The other wool attributes of length, tensile strength, etc., that influence the actual spinning power of wool are regarded as qualifying factors to fineness.

The coefficient of variability of fibre thickness ranges from 10 per cent. to 32 per cent. The bulk or 79 per cent. of the samples conform to a coefficient of variability of 16 per cent. to 24 per cent. There is no significant correlation between coefficient of variability and fibre fineness which demonstrates that coarse wools and fine wools are of equal magnitude as regards variability when coefficient of variability is the measure.

The coefficient of correlation between fibre fineness and crimping is a controversial topic among research workers. Some find an insignificant coefficient of correlation, others find the relationship definite. In general it appears that the workers who established a correlation took into account selected material such as well grown wools away from skin folds or shoulder samples of stud ram wools. The present study shows that where a large number of South African Merino samples are considered which include well grown wools, droughty wools, wools from skin folds, and from different regions of the sheep, a definite coefficient of correlation is established, though not a high one. In general, size or number of crimps per inch indicates coarseness or fineness in the wool fibres. The accuracy with which exact agreement between quality numbers on crimps and quality number on fibre fineness can be established is doubtful and crimping alone is a poor guide for estimating quality number. It is shown that only 28 per cent. of the samples have a perfect agreement between crimping and fibre fineness. Seventy-two per cent. may be out in the agreement of the two standards, some even to the extent of two to three qualities. Accurate estimations for quality number must, therefore, take into account fibre fineness rather than crimps. In the finer qualities of 80's and above an estimate of fibre fineness by hand and eye is often more difficult than in the case of the coarser Merino qualities and instances are recorded where the practical man has had to resort to laboratory analysis for an estimation of the finer Merino qualities.

The method of measurement of fibre fineness by the projection camera has been used by several workers. In the present study it was found preferable to gravimetric methods in that it supplied the analysis of fibre variability within the sample, and is a quick and ready method. Moreover, the mean fibre thickness obtained by the Camera method does not differ very appreciably from the results obtained by the gravimetric method. When a large number of estimations are necessary such as is often required in sheep experiments and routine analyses, the projection method was found preferable and has been used advantageously.

SUMMARY.

An anlysis of one thousand South African Merino wool samples selected at random and involving the measurement of a quarter of a million fibres is described and fundamental characteristics are demonstrated.

Fibre thickness ranges from $7\cdot5\mu$ to 50μ. The bulk, or eighty per cent. of the fibres measure from $12\cdot5\mu$ to $22\cdot5\mu$.

Eighty per cent. of the samples conform to the quality numbers of 60's to 100's. Eleven per cent. are 120's to 150's and it is suggested that these are "hunger fine" and presumably influenced by adverse conditions of nutrition. Eight per cent. are cross-bred and are coarser than Merino quality numbers.

As regards coefficient of variability, South African Merino wool ranges from 10 per cent. to 32 per cent. Seventy-nine per cent. of the samples conform to a variability of sixteen to twenty-four per cent. coefficient of variability.

The coefficient of correlation between fibre finess and coefficient of variability is given as $-\cdot 0754 \pm \cdot 0217$ and indicates an insignificant correlation.

The coefficient of correlation between fibre fineness and crimping is given as $-\cdot 617 \pm \cdot 0226$, which shows a definite correlation, though not a high one.

Twenty-eight per cent. of the samples show a perfect agreement between the quality numbers of fibre fineness and those of crimps.

The woolman who judges fibre fineness on crimps may err in seventy-two per cent. of his judgments by one, two or even three quality numbers.

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Section VIII.

Miscellaneous.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 3, Number 1, July, 1934.

Anatomical Studies, No. 41 : Prenatal Death in the Merino Ewe, associated with deformity of the Cervix Uteri.

By

H. H. CURSON, Dr.Med.Vet., F.R.C.V.S., Veterinary Research Officer, Onderstepoort; and

C. E. BELONJE, University of Pretoria.

The object of this note is to draw attention not only to the somewhat frequent occurrence of prenatal death in the pregnant Merino ewe, but also in this instance to an unusual dilatation of the *canalis cervicis* (Quinlan, 1929). Corner (1923) has undertaken a thorough investigation into intrauterine mortality in the pig.

The specimen in question (see figure) was obtained on 18th August, 1933, through the kind offices of Messrs. G. Pilditch and Coetzee of the Pretoria Abattoir.

As is well known, the fate of a dead foetus may be as follows: (*a*) abortion, or (*b*) maceration generally accompanied by pyometra and even pyosalpinx and cervicitis, or (*c*) mummification. In this case twins had been present and death had taken place at about 2 months.

Each of the ovaries showed the embedded corpus luteum and in one instance some retrogression had taken place. Only portions of the oviducts had escaped the slaughterman's knife and these were much thickened. The uterus contained both bones and pus, but most of the latter had filtered through to a dilatation of the cervix. The foetal membranes and maternal cotyledons had disappeared and the uterine wall throughout was greatly thickened. It may be added that the dressed carcase of the ewe was in excellent condition.

In regard to the deformity of the cervix (see Curson, 1932), this was not only greatly dilated, but it contained only three valves, an anterior, which was pervious, and two posterior. On incision 20 c.c. of liquid pus escaped from the cervical dilatation and in this were several bony fragments.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 3, Number 1, July, 1934.

Anatomical Studies, No. 42: Polydactylism in a Pig.

By S. J. VAN DER WALT and H. P. A. DE BOOM, University of Pretoria.

THE above condition was observed in June last at the Pretoria Abattoir and Mr. G. Pilditch, the Superintendent, kindly handed the specimen to the Teratology Class for description. It is interesting that the *fore* limbs were involved (Curson 1931).

The *left foot* bore medially an additional digit, which, for convenience will be named the first. It was, however, not only thicker and longer than the second digit, but it presented distally a vestigial structure to which reference will be made when describing the bony components. The digit in question was directed towards the lateral aspect of the foot and was slightly twisted so that the sole faced outwards. The manus otherwise appeared normal.

On removal of the soft structures (which were not studied) it was found that the manus except for the presence of the additional digit was normal.

The (first) digit possessed a well-developed metacarpus which articulated, as did the second metacarpus, with the first carpal bone. There was also present at the lateral aspect of the head a facet for articulation with the medial aspect of the head of the second metacarpus. Fused to the medial aspect of the third phalanx was a bone, which clearly represented the third phalanx of yet another digit. Articulating proximally with this was a small bone corresponding to a second phalanx but which in Fig. 1 gives, on account of it being broken, the impression that two bones are present. This small bone articulated with the second phalanx (of the first digit) by means of a small lateral facet.

The *right foot* bore in addition to the normal third, fourth and fifth digits, three digits on its radial side. Two of these, except for their slightly smaller size corresponded to the normal left third and fourth digits, and except for being a trifle shorter, gave the impression of forming the chief digits of another " foot ". A small digit, easily seen from the volar aspect of the foot, was wedged in between the normal and extra chief digits. This, in spite of its relatively small size, may be taken to represent the second digit of either the normal or extra " foot ". Dissection and removal of the soft structures, however, seemed to associate it more closely with the extra " foot ". See Fig. 2.

On studying the individual bones it was evident that the three supernumerary digits (the third, fourth and fifth digits were regarded as normal) could be viewed, as indicated above, as belonging to an additional " foot ". Instead of one digit, the second, being present, the metacarpals and bones of the third and fourth chief digits of a

left foot were to be seen. As mentioned above, the small supernumerary digit,(×) on account of its intimate fibrous attachment to the proximal part of the first phalanx of the third digit of the additional " foot ", might be considered to belong more properly to the extra " foot ".

Unfortunately the proximal row of carpal bones was not collected, but the first and second bones of the distal row were fused and the ventral surface provided an articulation for the proximal ends of the chief digits of the additional " foot ".

On the dorsal surface of the second phalanx of the third digit of the additional " foot " an exostosis was present.

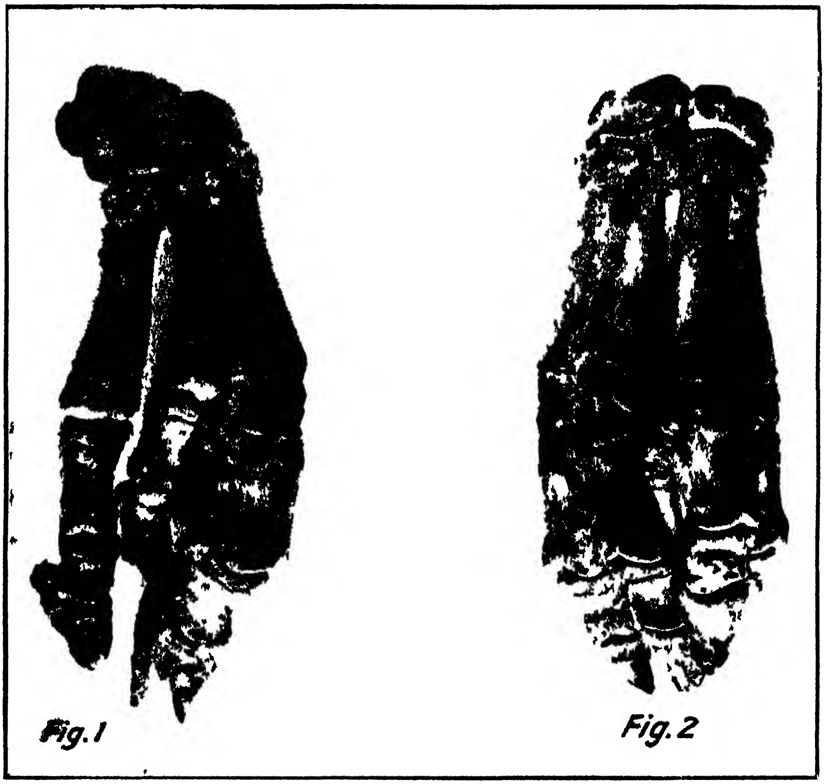

Fig. 1 Fig. 2

The position may be summarised by stating that the *left* foot possessed an additional (first) digit, and to the third phalanx of this was fused the rudiment of yet another medial digit. The *right* foot, besides having its normal chief digits and fifth accessory digit, showed medially three digits (two chief and second accessory digits) of a left " foot ".

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 3, *Number* 1, *July,* 1934.

Anatomical Studies, No. 43: Two Cases of Missing Incisor Teeth in the Horse.

By H. H. CURSON, Dr.Med.Vet., F.R.C.V.S., Veterinary Research Officer, Onderstepoort.

In a previous note (Curson 1930), a missing right permanent incisor in the upper jaw of a horse was reported. In this study a similar teratological condition will be described.

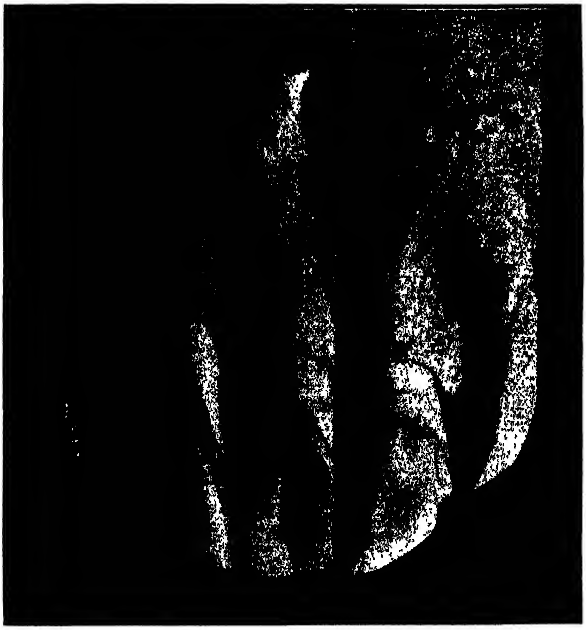

Fig. 1.

Case 1. *Horse* 20155.

This specimen was obtained by Dr. A. D. Thomas while conducting a *post-mortem* examination on 20th October, 1932. Horse 20155, shot on account of Horse Sickness (P.M. 11,168) was aged, being approximately 15 years old. The incisor teeth, labial view, are clearly seen in Fig. 1 and it would seem that the absent tooth was the left corner.

Case 2. Horse

In this case both corner incisors were missing, the age of the horse being approximately eight years. See Fig. 2.

I am indebted to Dr. J. Scheuber for this specimen, which was originally collected by Dr. E. Robinson, F.R.C.V.S., in 1920.

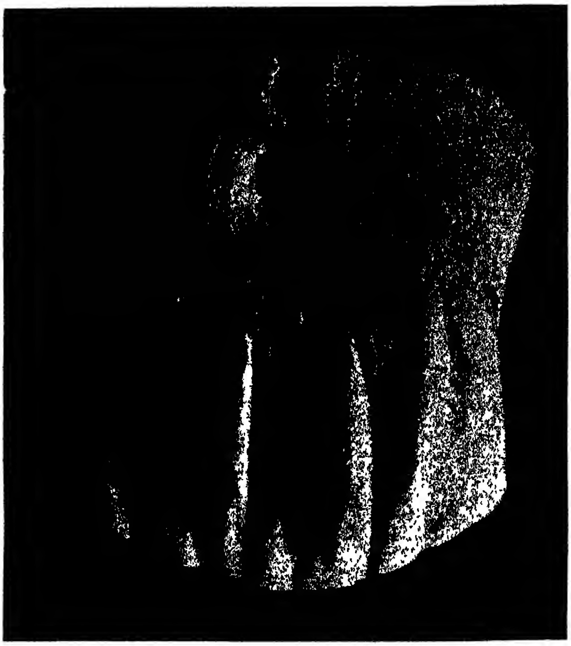

Fig. 2.

REFERENCE.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 3, Number 1, July, 1934.

Anatomical Studies, No. 44: On two anomalies arising from the Embryonic Small Intestine.

By H. H. CURSON, Dr.Med.Vet., F.R.C.V.S., Veterinary Research Officer, Onderstepoort.

WHILE anomalies of the small intestine are not rare in the domesticated animals, their infrequent occurrence nevertheless makes their record a matter of some interest.

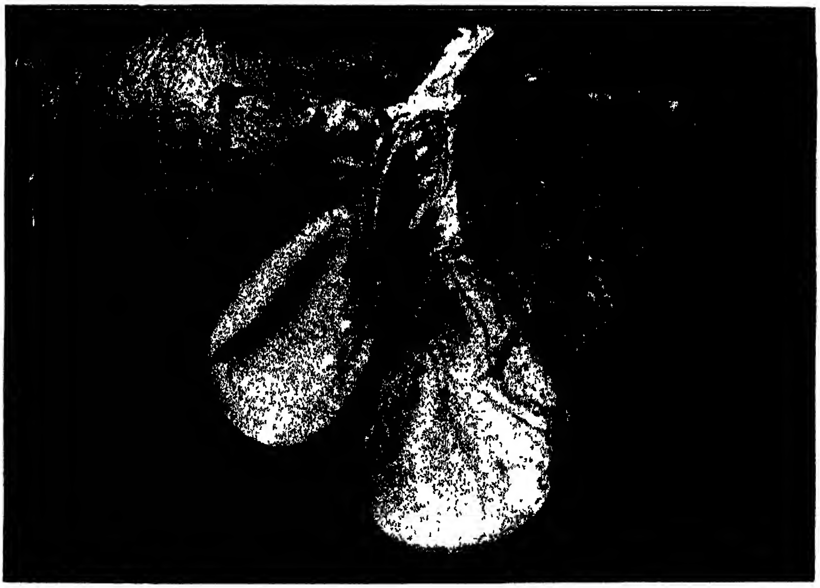

Fig. 1.

(*a*) *Duplication of the gall bladder in a pig.*—This specimen (see Fig. 1) obtained in 1925 from Dr. J. Botelho, Chief Veterinary Officer, Lourenco Marques, clearly shows the nature of the anomaly. The gall bladder, which (with its cystic duct) represents " a special offshoot of the early diverticulum " from the ventral aspect of the future duodenum, has subdivided into two. See also Fig. 2 which shows the anomaly in Sheep 31,700 (Path. No. 12,033).

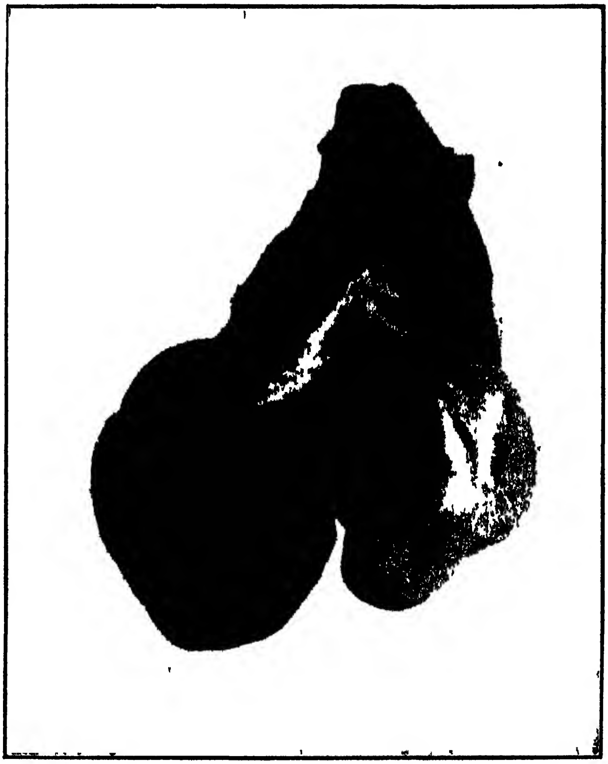

Fig. 2.

(*b*) *Jejunal diverticulum in a sheep.*—While diverticula of the intestines, especially the ileum (McMurrich and Tisdall 1928), are somewhat frequently recorded in the human, veterinary cases are comparatively rare. Sheep 20191, 6 tooth, which died on 27th September, 1928, of carbolic acid poisoning following dipping, showed on post-mortem examination a diverticulum of the small intestine 25 cm. long by 2.5 cm. wide at a distance of 156 cm. from the ileo-caecal valve. As will be observed from the accompanying photograph (Fig. 3) the jejunal cul-de-sac (A) is even longer than the caecum (B) which measured 19 cm. in length by 4.5 cm. in width. The presence of nodules of *Oesophagostomum columbianum* is also shown, especially at the initial portion of the colon (C).

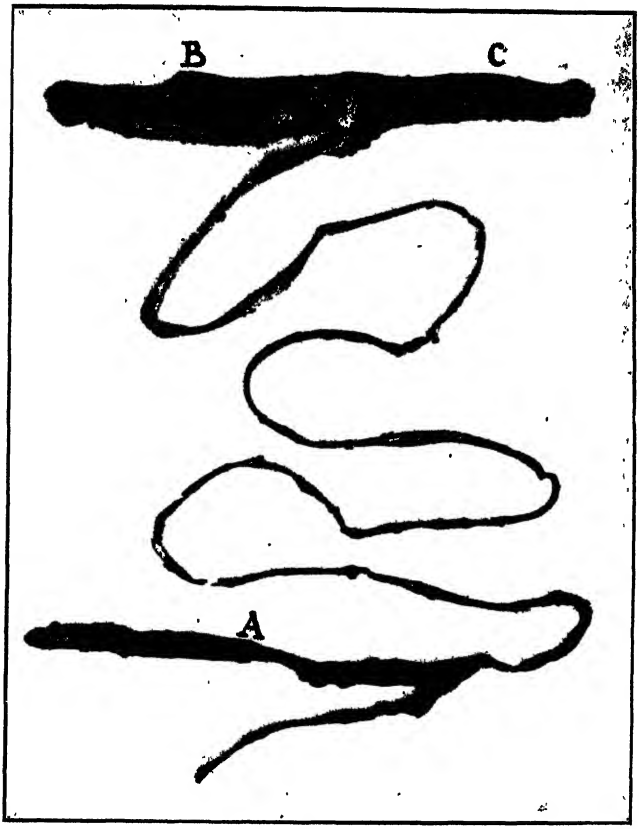

Fig. 3.

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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 3, Number 1, July, 1934.

Anatomical Studies, No. 45: Synotus in a Lamb and a Pig Foetus.

By H. H. CURSON, Dr.Med.Vet., F.R.C.V.S., Veterinary Research Officer, Onderstepoort.

AREY (1931) describes synotus as a rare condition in man. Several cases have been encountered in the domesticated animals, especially the sheep, and two are represented in the accompanying photographs, e.g. Figs. 1 and 2, Sheep; and Fig. 3, Pig.

Fig 1

The lamb (Persian) was born on 27.4.28 and lived for about an hour. It was received from Messrs. Rees, Shapiro and Behrmann, P.O. Meyerton, and thanks are due to this firm for the material (see File 125/221 and Path. No. 7998).

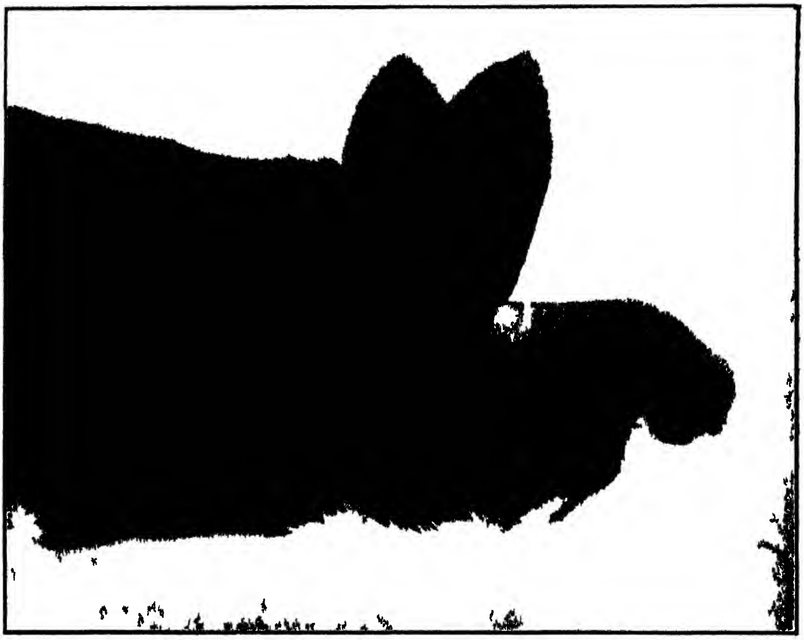


Fig. 2

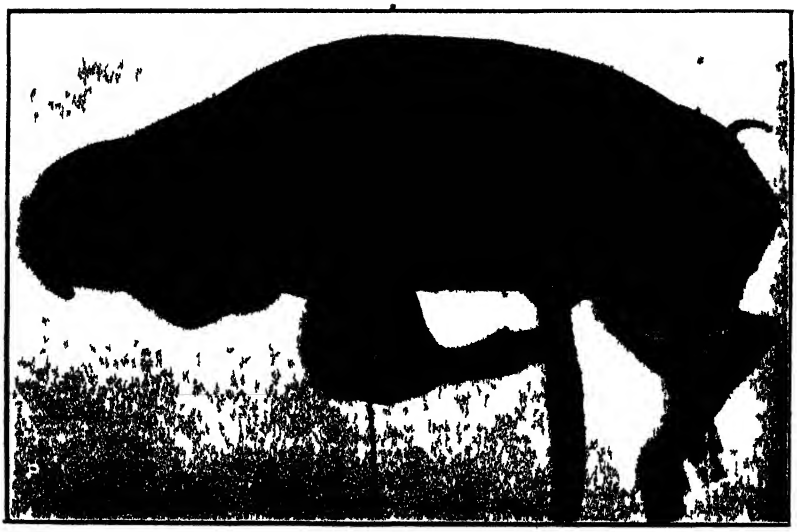


Fig. 3.

The pig foetus was obtained from the right horn (nearest one to cervix) of a large black sow on 2.9.27. The corpora lutea were situated as follows:—Right ovary 4, and left ovary 2, and the foetuses were, Right Horn 2 and Left Horn 3 (see Path. No. 7289). The other foetuses were normal.

It will be observed that in each case the ears are fused at their bases, and the nose is a proboscis-like structure. In the lamb the eyeballs are prominent, whereas in the pig they are absent.

The condition " illustrates the primitive location of the ear primordia before being wedged apart by the growing mandible ".

REFERENCE.

AREY, L. B. (1931). *Developmental Anatomy.* Wm. Saunders & Co., Philadelphia and London, p. 438.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 3, Number 1, July, 1934.

Anatomical Studies, No. 46: Apus in a Pig.

By H. H. CURSON, Dr.Med.Vet., F.R.C.V.S., Veterinary Research Officer, Onderstepoort.

THE condition of apus or absence of the pelvic limbs is exceedingly rare, and the fact that the subject of this note was able to move about with very little trouble is worthy of record.

Pig 585 female, born in April 1926, was presented to this Institution by Mr. D. King, P.O. Warden, at the age of three months, through the endeavours of my colleague, Dr. J. G. Bekker.

FIG. 1.

As will be seen in Figs. 1 and 2, the animal walked quite readily, the posterior part of the trunk being thrown upwards in the air, as a boy would do in walking on his hands. Fig. 1 shows the intermediate stage between the recumbent and the walking positions.

At the age of 18 months the pig was killed for anatomical purposes, but only the osteology was studied. Fig. 3 shows the mounted skeleton (see Path. No. 9293).

Fig. 2.

Photos showing the animal in question appeared in the *Natal Farmer* of 11th March, 1927, and correspondence relating to the pig is to be found in File 141/1346.

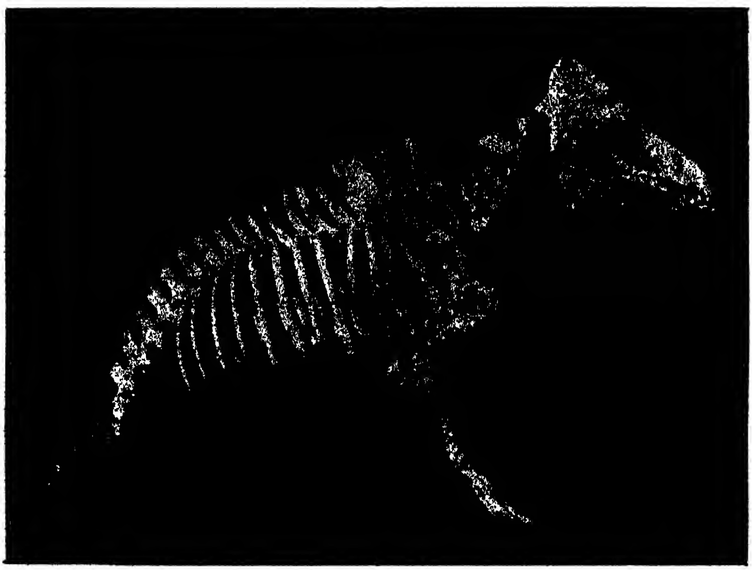


Fig. 3.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 3, Number 1, July, 1934.

Anatomical Studies, No. 47: Pervious Urachus in a Bull Calf.

By

I. P. MARAIS, B.Sc.Agr., B.V.Cc., Veterinary Research Officer, Onderstepoort, and

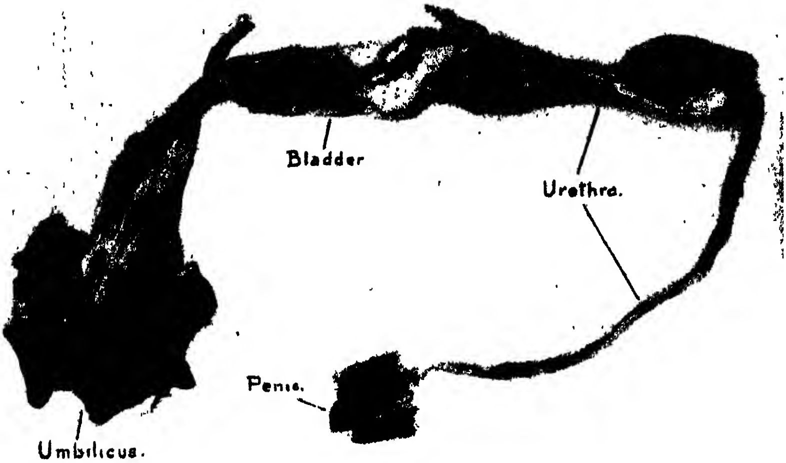
G. D. SUTTON, University of Pretoria.

The above condition, occurring in a Sussex bull calf D.O.B. 5434, was observed only a few days before death (31st August, 1933), the animal then being 5 weeks of age. The reason for this presumably is that the external urethral orifice in the male is adjacent to the umbilicus and more than a superficial examination would be necessary to diagnose the condition. When, however, the calf showed loss of condition a careful inspection revealed not only an abscess in the umbilical region, but also that a catheter would pass *per umbilicum* into the anterior end of the bladder and so release a quantity of pus-contaminated urine.

The *post-mortem* examination revealed not only cystitis but also pyelo-nephritis of the right kidney. Other secondary lesions (P.M. No. 12412) were catarrhal pneumonia of the right lung, purulent foci in the liver, and fatty degeneration of the myocardium.

Now at birth, as a result of the rupture of the umbilical cord, blood no longer flows through the umbilical vessels and the two arteries become the round ligaments of the bladder. Instead of the tube-like urachus (allantoic duct between bladder and umbilicus) becoming atrophied and being represented at the cranial end of the bladder as the middle ligament (*centrum verticis*), in the above case, the urachus remained patent and urine was therefore excreted not only by way of the urethra but also *via* the urachus. In the *post-mortem* report the fact that the bladder was described as tubular and extending forward to the umbilicus can therefore be understood. Incidentally the contents were dirty grey and floccular due to the pus. Owing to the pus infection at the umbilical orifice the tissues in this region were thickened. It is significant that the left round " ligament " was large and possessed a lumen of 0.5 cm. which, however, became obliterated as the vessel approached the cranial end of the bladder. The thickness of the wall was not constant, but thicker dorsally. The right round " ligament " was small and had no lumen, its diameter being 0.1 cm. The left ureter, too, was much larger than the right and had a lumen of 0.2 cm., whereas the lumen of the right ureter could only be determined with the finest probe. This is significant when the fact that the right kidney

contained pus is appreciated. A possible explanation is that after infection had extended to the right kidney *via* the right ureter, it ceased functioning and the right ureter accordingly atrophied. The left ureter now called upon to perform additional work became dilated. (See Figure.) It may be added that the urachus was ligatured two days before death.



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Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 3, Number 1, July, 1934.

Anatomical Studies, No. 48: On a case of Brachygnathia Superior in an Ox.

By H. H. CURSON, Dr.Med.Vet., F.R.C.V.S., Veterinary Research Officer, Onderstepoort.

THANKS to Dr. E. M. Robinson, F.R.C.V.S., the skull of red native ox, D.O.B. 5215, and killed 12/9/33, was handed to the Teratological Collection (Path. No. 14257). As will be seen from Fig. 1, the lower

Fig. 1.

jaw was undershot to such a degree that the first premolar (ox was about three years of age) was entirely unworn. The *pars molaris* of the *mandibula* was more concave than usual and in a corresponding manner the alveolar margin of the *maxilla* was convex. As is shown more clearly in Fig. 2 the anterior margin of the *premaxillae* is opposite the plane of the mental foramina.

Other changes involving the skull were observed on removal of the soft structures. The *ossa nasalia* were distorted, being directed towards the left of the median plane, the *vomer* was likewise bent and as the two " halves " of the *cavum nasi* were not equal, the *ossa turbinata* were correspondingly irregular, those of the right side being larger than those of the left. As can be imagined the bony floor of the nasal cavity was also involved, the width of the right side being greater than that of the left.

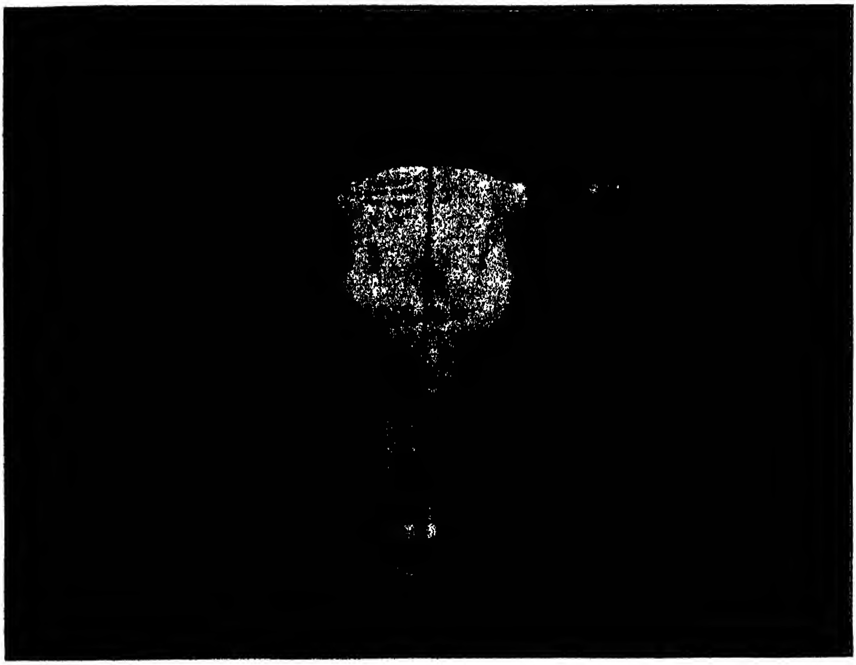


Fig. 2.

As indicated by Maré (1932), faulty jaws are a great hindrance to normal prehension and in the case in question the condition was very poor.

REFERENCE.

MARE, G. S. (1932). Anatomical Studies, No. 34. Faulty Jaws in Sheep. *18th Rept., Dir. Vet. Serv. and A.I.*, p. 1085.

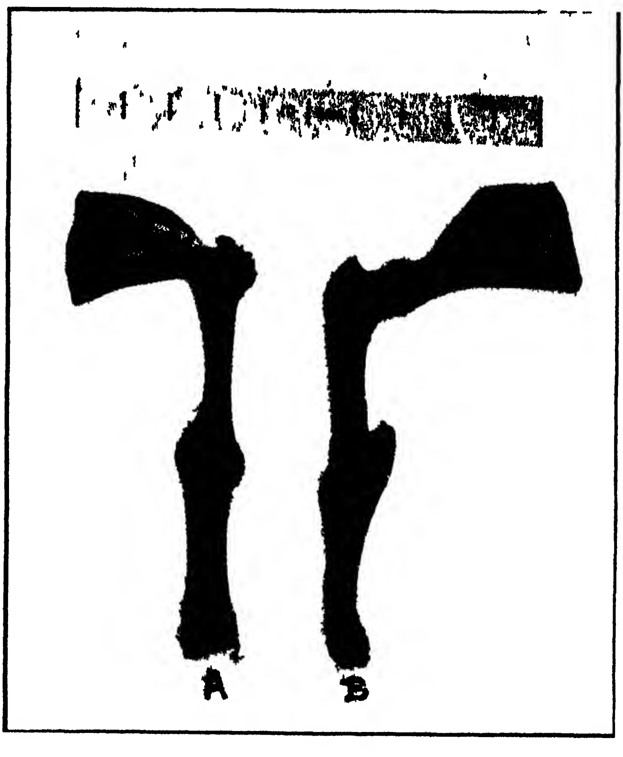
Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 3, Number 1, July, 1934.

Anatomical Studies, No. 49: On a deformity of the shoulder joint in a pig.

By H. H. CURSON, Dr.Med.Vet., F.R.C.V.S., Veterinary Research Officer, Onderstepoort.

On 5th October, 1933, the Large White Sow, D.O.B. 809, gave birth to nine piglets, all of which were normal, except one that was deformed and born dead.* This was the third litter of the sow and nothing unusual had occurred previously.

The piglet (Path. Book No. 14,295) on examination showed the following anomalies:—



* Sow served on 15/6/33 by Large White Boar, D.O.B. 901.

(*a*) The main lesion lay in the shoulder joint of the left thoracic limb. Later, on dissection, it was seen that the *angulus glenoidalis* did not bear a *cavitas glenoidalis*. Further, the humerus, instead of being directed the normal way, has its *facies cranialis* directed caudally. As a result the *antibrachium* and *manus* were also directed backwards. See figure.

Although the *articulatio scapulo-humeralis* possessed a joint capsule the lateral aspect of the *caput humeri* was attached to the *collum scapulae* by fibrous adhesions. Thus the joint instead of being diarthrodial was synarthrodial.

(*b*) There was also distortion of the *columna vertebralis* in the cervical region.

I am indebted to Mr. G. N. Murray, of Onderstepoort, for the specimen.

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Director of Veterinary Services and Animal Industry.

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Section I.

Protozoal Diseases.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 3, Number 2, October, 1934.

Eperythrozoon Ovis (sp. nov.) Infection in Sheep.

By

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THE demonstration by Biffi (1903), Gastiaburú (1903) and Barton (1905) of bacillary shaped bodies in the erythrocytes of patients suffering from Oroya fever and Verruga peruviana stimulated a great deal of work which has added immensely to the knowledge of the *Bartonella* group of organisms. Barton continued his observations and in 1909 reported that the intracellular organisms which he constantly found in patients were of the nature of protozoa and that he believed them to be the cause of Oroya fever or Carrion's disease. Subsequently these observations were confirmed by the American Commission consisting of Strong, Tyzzer, Brues, Sillard and Gastiaburú (1913). However, many other workers did not regard these bodies as parasites, and brought forward to disprove their parasitic nature arguments similar to those advanced to discredit the parasitic conception of the *Anaplasmata.* Not until Noguchi (1926) showed that the *Bartonella* could be cultivated was their parasitic nature accepted. Finally Noguchi and Battestini (1927) and Mayer and Kikuth (1927) showed that there was a common aetiological agent responsible for Oroya fever and Verruga peruviana, which frequently follows Oroya fever, viz., *Bartonella bacilliformis.*

Parasites similar to the *Bartonella* found in man have been described in animals. Mayer (1921) described *Bartonella muris* the cause of infectious rat anaemia. Kikuth (1928) demonstrated *Bartonella canis* in the dog and Schilling (1928) recorded *B. muris musculi* in the mouse. In several wild rodents various other species have been observed.

A common symptom produced by these organisms is an anaemia characterised by degenerative and regenerative changes in the blood. These changes occur sometime after the appearance of the parasites, in a manner similar to that observed after infection with *Anaplasma.* In fact, between Anaplasmosis and Bartonellosis there appears to be some relationship. The morphology and staining properties of the causal organisms are very similar, the clinical syndrome in each case is practically identical and in both conditions splenectomy is followed by a reappearance of the parasites in the blood.

Closely related to *Bartonella* is the genus Eperythrozoon. *E. coccoides* was first described by Schilling (1928) in splenectomized mice. Shortly afterwards Dinger working independently proposed the name *Gyromorpha musculi* for the same organism observed in splenectomized mice in Amsterdam. These observations were compared by Mesnil, Brynoghe, Vassialidis, Elliot and Ford. The latter recorded their occurrence in *Arvicola arvalis* and *Mus minutus* in addition to *Mus musculi*. Schwetz (1933) reported their occurrence in field mice in the Belgian Congo.

Schilling, Dinger and other workers found that 2–4 days after splenectomy of infected mice small ring-shaped organisms $0 \cdot 5\ \mu$–$1 \cdot 0\ \mu$ in diameter appeared in blood smears stained by Giemsa. Dinger described only ring forms but Kikuth in addition found rod-shaped and coccus forms. The parasites occur free in the plasma in addition to being found on the red cells. For example Dinger mentions that on the 2nd day after the appearance of parasites in splenectomized mice their number increases especially in the plasma where they may be 20–30 times as frequent as erythrocytes. On the 3rd and 4th day of infection large numbers can be seen lying on the red cells especially on the polychromatic erythrocytes. After a further interval of 24–48 hours the parasites may have disappeared but there is usually a periodic reappearance over a period of many weeks.

The course of *Eperythrozoon* infection in white mice differs markedly from that seen in field and wild mice. In striking contrast to the severe anaemia seen in infectious rat anaemia produced by *Bartonella muris*, white mice do not appear sick and any anaemia definitely is not severe. On the other hand, Kikuth in his work on wild mice observed severe clinical symptoms and even death in some of his animals. After experimental infection he observed anaemic changes characterised by erythro-phagocytosis and the appearance of numerous normoblasts. An extraordinary phenomenon was the extreme fluctuation in the number of parasites in this respect the behaviour closely resembling that of *Bartonella canis*. This periodic disappearance and reappearance of parasites might be observed 4 or 5 times. When the parasites were rare ring forms could be differentiated easily from rod and coccus forms but when the parasites were frequent Bartonella forms were found almost exclusively. This variation in the number of parasites together with the production of severe clinical symptoms anaemia and even death led Kikuth to believe he was working with a mixed infection of *Eperythrozoon coccoides* and *Bartonella muris musculi* but on subinoculation into splenectomized white mice these animals showed no clinical symptoms and only ring forms were encountered.

In spite of some difference in morphology, in location of the parasites, and in the clinical picture produced by infection with *Eperythrozoon* and *Bartonella* it must be concluded that the two parasites are closely related. There is no conclusive evidence for the acceptance of *Eperythrozoon* as a parasitic entity and not as a product of desquamation but the transmission from infected to susceptible white mice and rats, their reappearance after splenectomy, and the chemotherapeutical influence of neosalvarsan in sterilising the blood is significant.

Observations at Onderstepoort.

During the course of experimental work on Heartwater (*Rickettsia rummantium* infection) 10 c.c. of blood from a sheep at the height of the Heartwater reaction was injected into a splenectomized sheep. Four days later peculiar ring-shaped bodies $0 \cdot 5\ \mu$–$1 \cdot 0\ \mu$ in diameter were observed in blood smears from this sheep stained with Giemsa. At first these bodies were regarded as stain deposit or artefacts but each of these possibilities was ruled out by taking meticulous care with the cleaning of slides and the preparation of the blood fibres, and by staining with filtered stain in the inverted position. The bodies which were to be seen on the red cells as well as free in the plasma, increased in number daily. On the fifth day, i.e., several days before the "virus" of Heartwater could be expected to be found in the peripheral circulation, blood was subinoculated into three normal sheep. The peculiar organisms were found in each of these sheep from the 7th day, and were carried on for several generations by repeated subinoculations. The original splenectomized donor succumbed to Heartwater on the 13th day after injection; none of the subsequent generations of sheep developed Heartwater to which disease their susceptibility was demonstrated later by immunity tests.

Smears were prepared daily from each of the subinoculated sheep and from these preparations stained with Giemsa it is possible to give an accurate description of the peculiar parasites. It is now apparent that these organisms had been observed previously on many occasions but their significance had not been appreciated fully.

Description.

The organisms may be found lying supracellularly on the erythrocytes but the greater number are to be found lying free between the cellular elements in the blood smears.

Position. (*a*) *The supracellular forms.*—The number of parasites lying on the erythrocytes varies within very wide limits, but usually the number of supracellular forms is directly proportionate to the intensity of the infection in the blood. A single organism may be present or alternately the entire surface of a red cell literally may be covered with parasites which may there be lying on top of one another. The most common occurrence is for the supracellular forms to be found in clusters of from 3 to 12 aggregated towards the centre of the cell, or at a point towards the periphery or along a portion of the border.

(*b*) *The extracellular or free forms.*—Invariably the free forms are the more numerous. As will be seen from the photographs at the end of the text, they are fairly evenly distributed in dessiminated groups throughout the preparations.

Morphology.—Typically the organisms appear as delicate rings approximately $0 \cdot 5$–$1 \cdot 0\mu$ in diameter. Fixed with May-Grunewald and stained with Giemsa they take on a delicate pale purple colour. In the ring-forms about 4/5 of the centre portion fails to take the stain and they appear to be flat, not spherical. They predominate both supra- and extracellularly but can but be studied in those

portions of a smear where clumps of the cells are widely separated. In addition to the ring forms there are frequently to be seen ovoid forms, irregular triangles with the angles rounded off, in addition to rod, dumbell, and comma shaped forms.

A point of interest is that it has been noted frequently that at one end of a smear ring forms predominate while towards the other end rod and comma shaped forms are more frequent. It is assumed that this distribution is purely mechanical and may be the result of injury during the process of drawing the film.

In some of the ring forms there may be noticed one, two or even three points which stain an appreciably darker colour. Their significance is quite obscure but they may stand in some relation to multiplication.

Symptomatology.—Up to the present the disease has only been studied in Merino sheep under stable conditions. As yet nothing can be said about the cause of infection in other breeds of sheep, or in animals subjected to adverse conditions in the field, where it is assumed that the symptoms would be more pronounced and where the disease may be of some economic importance. In addition nothing whatever is known of the natural mode of transmission.

After artificial infection with blood by the subcutaneous or intravenous route the period of incubation usually varies from 4–15 days, but it may be extended up to several weeks. The first symptom is fever, the temperature rising to about 105° F., though it has been observed to rise as high as 107° F. The febrile reaction may be continuous for 3 or 4 days, or it may be intermittent. Febrile exacerbations and remissions at intervals of a week or more are common or alternately there may be a complete absence of any hyperthermia.

Parasites make their appearance concurrently with the first rise in temperature or they may be seen only a few days later. They then multiply rapidly and within a week may be 25 to 100 times as numerous as the erythrocytes. From the limited number of animals examined it would appear that active multiplication of the parasites continues up to the time when the first signs of anaemia make their appearance in the smears. Then the number of parasites suddenly decreases and when the anaemia is at its worst comparatively few organisms are to be seen. When the condition of the blood tends to become normal again, usually after about 4 weeks, there may be a recrudesence of infection again. This irregular fluctuation in the degree of infection may continue for 6 weeks or longer.

While the fever curve is not very characteristic for the disease the anaemia is a constant and regular symptom. Clinically it may be demonstrated 5 to 8 days after the first appearance of parasites in the smears. As the condition progresses the visible mucous membranes become more and more pale until eventually they have the appearance of white porcelain. A slight icterus has been observed in a few instances. Examination of the blood shows a rapid drop in the red precipitate on centrifugation and the red blood count may drop as low as 1,500,000 within 10 days. At the same time there is a rise

in the leucocyte count up to about 20,000. This increase is due chiefly to an absolute and relative monocytoses and erythrophagocytosis is a common feature. With the development of anaemia the results of degenerative and regenerative processes make their appearance, namely anisocytosis, polychromasia, punctate basophilia, jolly bodies and nuclear rests in addition to normoblasts. Provided no relapse occurs the blood picture returns to normal in about 4 weeks.

Other clinical symptoms are those of fever and anaemia and vary with their severity. One notices dullness, inappetence, loss of condition and debility. The pulse is rapid and weak and the respirations accelerated.

A fatal termination has not been noted and the prognosis under stable conditions is good.

Discussion.

Since the recognition of the above described organism as a parasitic entity, it has become apparent that certain febrile reactions and anaemic changes in the blood of sheep, which were inexplicable at the time, may now be accounted for. The ease with which the parasite may be transmitted by artificial means in the laboratory must serve as a warning to research workers· particularly those engaged in haematological studies of other infectious diseases. Whether the disease is of any importance from an economic point of view in the field still remains to be determined but it appears not unlikely that the extreme and prolonged anaemia may be of importance in reducing the natural resistance of affected animals to other conditions which alone may not be fatal. This aspect of the problem together with the natural mode of transmission is receiving attention.

A consideration of the parasite, its morphology, location, staining reaction with Giemsa and the nature of the disease produced, has led the authors to believe that it is a hitherto undescribed species of the genus *Eperythrozoon* for which the name *Eperythrozoon ovis* is proposed.

Classification.—*Eperythrozoon* bears an unmistakable resemblance to *Bartonella* and *Grahamella*. In many respects it appears to have a fairly close relationship with *Anaplasma* as well as was noted above. It is interesting to note the morphological differences between the type species of these four genera. *Anaplasma* consists of grains of chromatin, round oval or somewhat sharply irregular in shape. They are intracellular though occasionally they give the distinct impression of being epicellular and there are invariably only 1 or 2 (never more than 3 or 4) parasites in each erythrocyte. *Grahamella* consists of fairly regular rods which are intracellular and are always present in fairly large number (8–20) in the same cell. *Bartonella* is pleomorphic being represented by cocci, rods, rings and various irregular shapes. Their position is intracellular and they are always present in fairly large numbers. *Eperythrozoon* is also pleomorphic although the variety of shapes is probably not so great as in the case of *Bartonella*; ring and rod shapes predominate,

and the infection may be very heavy. The chief difference between these two genera is the situation of the parasites which in the case of *Eperythrozoon* is epicellular or extracellular.

As regards the result of infection with these different genera. *Anaplasma* in cattle causes a very severe disease characterised by extreme anaemia and the condition is frequently fatal; *Bartonella* in man is responsible for a serious condition (Oroya fever and Verruga peruviana); *Eperythrozoon* appears to result in nothing more serious than the production of fever followed by a severe anaemia. *Grahamella* is non pathogenic.

In view of this obvious relationship between these four genera it is proposed tentatively that they should be included in the family *Anaplasmidae*. This family which most protozoologists would presumably place in the order *Haemosporidia* could be defined as follows:—

Small blood parasites round, oval, rodshaped or irregular in shape whose average size is 0·5–1·0μ. They lie either in or on the red cells or occur free in the plasma. They may be non-pathogenic or pathogenic, in which case they most requently produce fever followed by a variable degree of anaemia. Transmission takes place probably in all instances by arthropods.

In passing it may be mentioned that Rickettsia has many features in common with the above group or organisms but its affinity for endothelial cells and its almost complete absence from the blood stream microscopically would seem to indicate a more distant relationship.

Summary.

1. A brief review of a portion of the literature on *Bartonella*, *Grahamella* and *Eperythrozoon* is given.

2. A new species of blood parasite of sheep is described for which the name *Eperythrozoon ovis* is proposed.

3. The symptomatology of the disease produced by *Ep. ovis* is detailed.

4. The tentative proposal is made to unite the four genera *Anaplasma*, *Grahamella*, *Bartonella* and *Eperythrozoon* into one family the *Anaplasmidae*.

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ADDENDUM.

Neitz and Quinlan found a species of *Eperythrozoon* in splenectomized calves at Onderstepoort, which were known to be carriers of *Anaplasma centrale*. After the operation *Eperythrozoon* appeared in extremely large numbers, and also *Anaplasma centrale* were present. At prsent it can not be stated whether the *Eperythrozoon* of cattle is identical with *Eperythrozoon ovis* of sheep, but transmission experiments to clear up this point are being undertaken.

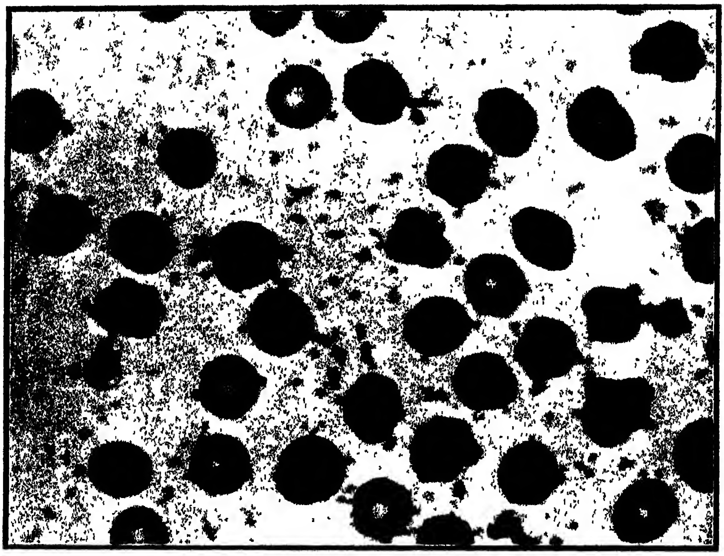

Fig. 1.—*Eperythrozoon ovis* of sheep. Numerous parasites lying free, 4th day of infection. Magnification 1,750 ×.

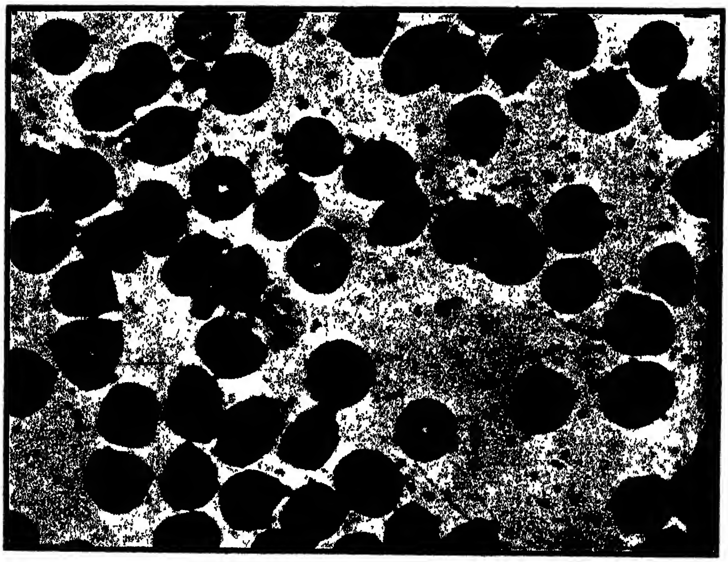

Fig. 2.—*Eperythrozoon ovis* of sheep. Some parasites are free and some supracellular. Magnification 1,500 ×.

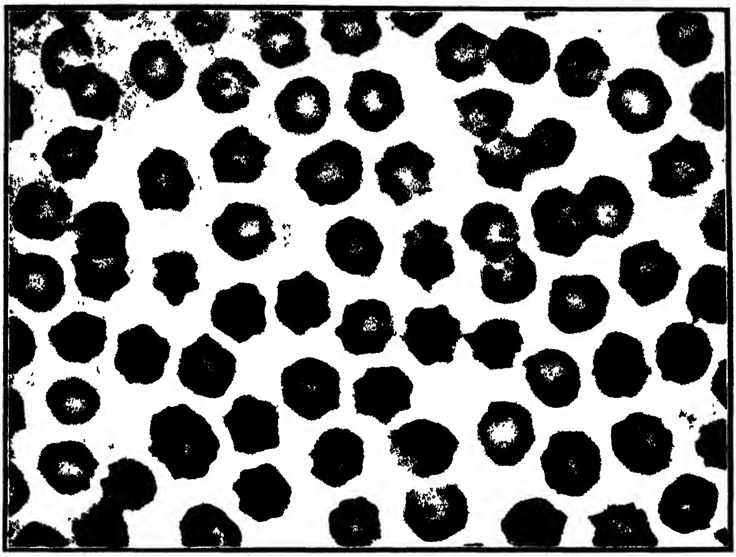

Fig. 3.—*Eperythrozoon ovis* of sheep. Parasites lying supracellular. Magnification 1,750 .

Fig. 4.—Anaemic changes due to Eperythrozoon infection. (Anisocytosis and punctate basophilia.) Magnification 1,500 ×.

Section II.

Virus Diseases.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 3, Number 2, October, 1934.

Investigations into the Transmission of Horsesickness at Onderstepoort during the Season 1931-1932.

By

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THE natural transmission of horsesickness, an extremely important disease of equines in South Africa, is not known up to the present, although more than 30 years have elapsed since the first experiments were commenced. Although the horse has to a great extent been superseded by mechanical transport to-day, the practical importance of the disease has not been greatly diminished since those early days.

During the summer of 1931-1932 we started a new line of research into this problem, which had held the interest of the Onderstepoort Laboratories for more than 15 years.

There were only a few facts, representing general opinions about horsesickness, at our disposal which could serve as a guide for these investigations, viz., horsesickness is not contagious; the disease does not spread by close contact in stables; its occurrence under natural conditions is restricted normally to the summer months and the severity of the outbreaks depends mainly on the amount of rain, in dry seasons few cases occur, whereas in wet seasons the incidence of infection is very high; infection seems to occur mainly at night, round about sunset or sunrise; stabling of animals itself affords fairly good protection and, furthermore, the tops of hills may be regarded as fairly safe; after the first good frost the disease disappears suddenly and no new cases make their appearance.

This information is naturally very meagre wherewith to locate the natural transmitter of the disease. It serves to indicate to a great extent, however, that mosquitoes are the most probable transmitters, and with this theory a satisfactory explanation of all the facts known up to the present could be advanced. Whatever the actual vector may prove to be, any work on the transmission of horsesickness has to begin, in our opinion, with mosquitoes.

For the carrying out of this work we had at our disposal, to start with, only one summer, viz., that of 1931-1932. Six months, even under the most favourable conditions, is a very short period for work of this nature, and we could consequently not expect to finish in the time. A mosquito survey, the result of which has been described in the first paper of this series, had to be conducted in order to obtain some indication as to the most probable vectors, as it was obviously impossible to experiment with every species of mosquito known to occur in South Africa.

Suitable methods for feeding the mosquitoes on horses and also for keeping them alive for a sufficiently long time had to be devised, the former work in this direction having failed mainly on account of technical difficulties. The actual experiments had then to be carried out in which, apart from the vector being unknown, the length of the incubation period of the virus in the insect host, the range of temperature in which its development could take place, and the suitable time for feeding the probable transmitters on the infected animals, were also all unknown.

The strain of virus we had to do most of our experiments with was an old laboratory strain, and only towards the end of our work did we succeed in obtaining material from some natural cases.

Furthermore, the climatic conditions were so adverse that our difficulties were still further increased. It was very often impossible to obtain the material we wanted.

We were not successful in finding the actual transmitter of horsesickness. We believe, however, that the negative results are also of some value, and we wish, therefore, to give a detailed account of our experiments and observations as this work will serve as the basis for future work on the same lines, and may be of value to other workers interested in the same problems.

I. GENERAL OPINIONS CONCERNING THE EPIDEMIOLOGY OF HORSESICKNESS. REVIEW OF LITERATURE.

The main epidemiological facts known up to the present which can be of some assistance in trying to trace the transmitter of horsesickness, have already been mentioned briefly. They were regarded as all pointing to some species of mosquitoes as the most probable carriers.

The insect transmission theory does not find much support amongst the farming community mainly on account of the lack of knowledge on the biology of insects and the part played by them in the transmission of other diseases. The farmers' observations are, however, often remarkably accurate, and theories of particular interest formulated by them may be encountered. These theories all correlate to a greater or lesser extent the disease in question with climatic and telluric factors as may be seen from the following examples.

The Dew Theory.

Observations on the actual time at which infection is most likely to occur has revealed the fact that early morning or late evening must be regarded as particularly dangerous. At this time the grass is generally covered with dew during the horsesickness and bluetongue season, and many farmers have come to the conclusion that it is actually this dew which is responsible. This to some extent supports our present knowledge of the habits of the particular varieties of mosquitoes under suspicion which are largely confined to moist localities such as those afforded by dewy grass and which, furthermore, are most aggressive under these conditions coupled as they generally are with the absence of wind, which greatly facilitates matters for the mosquito who is not a strong flier.

Toxin in Vlei Grass.

It has been noted that horsesickness and bluetongue are generally confined to low lying and marshy ground and that animals pastured on elevated ground are much less liable to infection. This has led to formulation of the theory that some toxin is developed in grasses in these low-lying areas under certain conditions of soil and climate, which is capable of setting up the diseases. This again bears out the mosquito theory, as these insects are to a very great extent confined to such localities as will be shown later.

Water.

Water has frequently been blamed, vague referenes to contamination, presumably of animal or mineral origin, being advanced as the cause. Here again a comparison similar to the preceding can be drawn.

Morning and Evening Mists.

This is an extremely old conception, the cause of human malaria having been ascribed to such mists. Nevertheless, it still persists in the minds of many even to-day. Under such an environment again mosquitoes find particularly suitable conditions.

A complete review of these epidemiological facts has been given by *Knuth* and *du Toit* in their textbook on tropical diseases of animals, where further details may be found. These authors came to the conclusion as well that mosquitoes are the most probable transmitters.

As far as we are able to ascertain the only actual transmission experiments with mosquitoes were carried out some 30 years ago by *Pitchford* (1902) in Natal. He claims to have obtained six positive results with unidentified Anophelines. He placed an infected horse together with a number of Anopheles in an " infection box ". After its death it was replaced by a normal horse. The resulting infection did not kill the horse but resembled a mild attack of horsesickness. It is not possible to obtain a very accurate idea of his experiments, as no satisfactory records are given. Only in one case is a temperature chart added. This horse had remained in contact with mosquitoes for 4 days, and 12 days later the temperature commenced to rise. Blood was subinoculated into another horse and three days

later the reaction appeared and the horse died on the 17th day, p.i. In another case the mosquitoes had been infected less than 48 hours previous to their feeding on this animal. This would suggest a mechanical transmission.

By some authors outside the Union arthropoda other than mosquitoes have been suggested as possible transmitters. No actual experiments have been carried out with them however. *Williams* (1913), *Leger* and *Teppas* (1922) and *Monfrais* (1923) regard *Lyperosia* species as probable transmitters, *van Saceghem* (1918) *Culicoides* and *Tabanus* species. The possibility of *Culicoides* acting as carriers is also discussed by *Patton* (1920). Furthermore, *Rockmann* (1911) and *Reinicke* (1912) for epidemiological reasons, regard ticks as the most probable transmitters, a theory which, however, does not agree well with those epidemiological facts mentioned in this paper and accepted for the time being as correct.

II. SCHEME OF EXPERIMENTS.

For reasons already stated in full, mosquitoes were regarded as the most probable transmitters of horsesickness and the mosquito survey carried out at Onderstepoort concurrently with the actual experiments, had pointed out that, disdegarding Anophelines owing to lack of information, species of *Aëdes* fulfilled most accurately the requirements of the known epidemiological factors. Representatives of this genus were, therefore, mainly used in the following experiments. The following were regarded as the most promising species; *Aëdes caballus, A. lineatopennis, A. hirsutus* and to a somewhat lesser extent *A. vittatus* and *A. dentatus*.

When the experiments were commenced in the later part of the winter of 1931, *Aëdes* species were not yet available and *Culex theileri* was the only mosquito obtainable in fair numbers. With this species a number of experiments were carried out, mainly to elaborate the experimental technique as far as possible before the actual season commenced. Furthermore, it seemed worth while experimentally to exclude this most common mosquito species from the list of possible transmitters. The transmission of horsesickness by insects might be direct, mechanical or indirect, requiring a certain multiplication or development of the virus in the insect's body.

That the direct or mechanical transmission is possible has been proved by *Schuberg* and *Vuhn* (1912), who succeeded in transmitting the disease in this way by *Stomoxys calcitrans*. Although mechanical infections may occur in nature, it is very unlikely that this is the ordinary way; e.g. daylight biting insects, especially *tabanids* and *Stomoxys* seem to be the most capable mechanical transmitters according to our knowledge of other diseases. Horsesickness is, however, normally not transmitted during day-time, as we have seen before.

Assuming the necessity for a multiplication or an actual development of the virus, a certain period will elapse before the insect is capable of transmitting an infection. In Yellow Fever, a human disease transmitted chiefly by *Aëdes* species, which closely resembles horsesickness in many respects, this period is at least 8 days and on an average about 12 days.

In our experiments the mosquitoes were injected into susceptible horses five days and longer after their infective feed on a virus horse, or fed after a period of at least 14 days. During the interval they were fed on sugar water in the laboratory.

A positive result after injection of mosquitoes would merely show the presence of virus in some part of the insect's body, but does not reveal anything about its capacity for transmitting the disease actually. This can only be proved by feeding experiments. On the other hand, a negative result obtained by injection is more definite than is the case in a feeding experiment, especially if the latter is carried out with a limited number of specimens.

The injection experiments therefore give quite a lot of useful preliminary information about the suitability of a species for transmission. A negative result obtained with mosquitoes 5-7 days after their feeding on a virus horse, is a strong indication that the species used is not a transmitter, whereas a positive result, although requiring confirmation by feeding experiments, may be regarded only as promising. The one method therefore lends a sense of completeness to the other.

In the feeding experiments a period of 14 days, after feeding on a virus horse, was regarded as sufficient to allow for the necessary development or multiplication of the virus in the mosquito.

It was not possible in our work to adhere to a programme planned beforehand, as we were not sure about the suitability of the strains of horsesickness at our disposal for this work, and as we depended to a great extent in the choice of our material upon the climatic conditions.

III. STRAINS OF VIRUS AND ANIMALS USED IN THE EXPERIMENTS.

In all, the following four strains of horsesickness virus were used in our experiments.

(*a*) *O-virus.*—The strain of virus used in the first series of our experiments consisted of the laboratory vaccine strain known as O-virus. This strain had originally been isolated from a case of horsesickness by Sir Arnold Theiler in the year 1901 and was subsequently chosen by him as the strain most suitable for immunization purposes.

In August, 1901, the first injection was made into horse No. 96, blood from this horse constituting the first generation of the virus. By repeated subinoculations into susceptible horses the strain was preserved and is to-day used in the preparation of the horsesickness vaccine being now in its 225th generation. The virus has proved to be of a very high grade of virulence, resulting almost invariably in fatal cases of horsesickness upon inoculation. From a perusal of the temperature charts of the horses used in its maintenance it appears that slight though definite alterations in its potency have taken place. The incubation period of the reactions produced by it have shortened somewhat, viz., from 4 to 6 days to the usual 2 to 3 days, as is the case to-day. The duration of the disease itself,

which was originally in the neighbourhood of 6 to even 10 days has been reduced to an average of 3 days and in some cases even less. It would appear, therefore, that some alteration in the virus itself has taken place, and coupled with the fact that this virus has had no opportunity of developing outside the body of horses throughout the 31 years from its recovery from a natural case of horsesickness, it seems quite reasonable to assume that some biological change has taken place which may have affected its propensity for developing in the body of the supposed invertebrate host.

(*b*) *Losperfontein Virus.*—This virus was obtained on 3rd March, 1932, on the Government Irrigation Settlement at Losperfontein, approximately 40 miles west of Pretoria, from a fatal case of horsesickness in a mule. Owing to some confusion in the records of the settlement office, a certain amount of doubt exists as to the accurate history of this case. So far as can be ascertained the mule

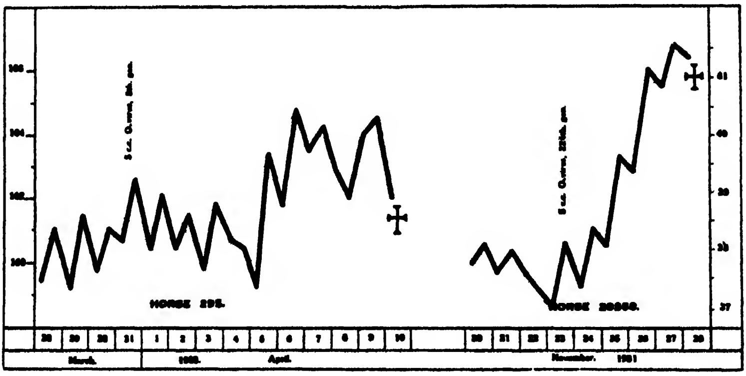

Fig. 1.—Comparison between horsesickness reactions produced by O-virus: 8th and 224th generations.

was immunized against horsesickness during the month of December, 1931, by the Onderstepoort method of hyperimmune serum and virus, one of the viruses used being the O-strain. The possibility therefore exists that we were dealing with a case of relapse after a period of three months, and had recovered the O-strain virus wherewith the mule had been immunized in December. It must be pointed out, however, that had this mule not acquired immunity or not been immunized in the first instance, the latter also being possible on account of the confusion amongst the records, chances for natural infection on Losperfontein were excellent. A perusal of the meteorological records revealed the fact that fairly good rains had fallen early in February, i.e. about one month previously. The mules had been running in a paddock containing a stream where the chances for mosquitoes breeding out were very good indeed. Natural infection can, therefore, not be lost sight of and is in fact extremely likely. However, the possibility does exist that a relapse might have occurred, in which case O-virus would have been recovered, and its difficult propagation in our experimental mosquitoes would then not be surprising assuming that O-virus has become biologically altered.

(c) *Eshowe virus.*—This strain of virus was obtained by the Government Veterinary Officer of Eshowe, Natal, from a mule suffering from horsesickness which ended in recovery. This mule had been immunized against horsesickness on the 25th August, 1931, by the serum-virus method.

In this case again the possibility of a relapse exists, after an interval of 6 months. The fact that this mule recovered seems to point to some other virus strain being responsible for setting up the infection, as O-virus is almost invariably fatal. This does not, however, constitute sufficient evidence to exclude the relapse possibility as cases of recovery from O-virus are known and further the mule may have had some natural immunity.

(d) *Kaalplaas virus.*—The Kaalplaas virus used in our experiments was obtained from a horse No. 20031 owned by the laboratory which had been running on the laboratory farm Kaalplaas, about 5 miles from Onderstepoort. This horse had been hyperimmunized against horsesickness, O-virus strain, 225th generation, in September, 1931, receiving 10,000 c.c. virulent blood from the donor, horse No. 20154. The animal was then sent to Kaalplaas where it was exposed to natural infection from 6th October, 1931, to 13th April, 1932, and contracted horsesickness on the latter date. It was returned to Onderstepoort, where a typical case of Dikkop horsesickness, which ended fatally, was noted. Considering that this was the only case of horsesickness amongst a large number of immunized horses with which this animal was running, itself hyperimmunized against the disease, it appears extremely likely that we were dealing with a case of relapse to O-virus.

It will be realized from the above that in the case of the three viruses collected from the field for experimental purposes, viz., Losperfontein, Eshowe and Kaalplaas, a history of immunization against horsesickness accompanies each case so that the possibility of our having dealt with O-virus throughout our experiments can, therefore, not be ignored.

(e) *Experimental Animals.*—The horses used in our experiments consisted of old cast animals of little commercial value bought by the laboratory for experimental purposes and for the production of horsesickness vaccine. The animals are recruited principally from the large towns, viz., Johannesburg and Pretoria, where they had been used for various purposes, e.g. hauling commercial vehicles, etc. No guarantee of any sort can be obtained as to their susceptibility or resistance to horsesickness nor is anything of their past histories known. The fact that the greater number of these animals have spent their lives in towns indicates that they have probably not been exposed to infection and from past experience it is known that only very occasionally is an immune horse encountered.

IV. EXPERIMENTAL TECHNIQUE.

The technique used for feeding mosquitoes on horse and keeping them alive in the laboratory for the periods required by the experiments, have been described in the second paper of this series, and we, therefore, refer the reader to it for full particulars.

From this paper it will be seen that the technique was gradually altered and improved. Different methods were therefore used in the different sections of this work. In the introduction to each section we will briefly refer to the methods actually applied in that particular part.

V. EXPERIMENTS WITH O-VIRUS.

We commenced our experiments with the O-virus laboratory strain, which was isolated, as previously stated, more than 30 years ago, and had been transmitted through about 200 generations by intrajugular injection from horse to horse.

As mentioned previously, this strain has changed during these 30 years, especially the incubation period, which has considerably shortened, being at present only 2-3 days. The strain has virtually acquired the character of a virus fixe, and it is, therefore, not unreasonable to suspect a change in other biological respects as well.

From the commencement we had some doubt about the suitability of the strain for transmission experiments. It seemed possible that the exceptionally long direct transmission of the strain from horse to horse could have affected the developmental capacity of the virus in its natural insect host.

In the literature no reference is given to similar biological alterations in respect of other virus diseases of man or animals transmitted by insects, but no other virus, to our knowledge, has ever been transmitted through a series of direct transmissions by means of the syringe only. We know, however, that in trypanosomiasis the developmental capacity of the parasites in tsetse flies is very quickly reduced and lost through direct transmission. On the other hand, the human malaria parasite is not affected by such a procedure.

In connection with the theory of a reduced developmental capacity of the virus in its invertebrate host, a recent outbreak of horsesickness at Wellington, Cape Province, may serve to throw some light upon the subject. For a number of years this area was regarded as free from horsesickness. On 3rd March, 1931, some horses were immunized against horsesickness with the Onderstepoort strain. On 29th March one of the non-immunized contact horses died from horsesickness and shortly afterwards several others followed.

These cases seemed to indicate that the virus was still capable of being transmitted in the natural way as we are forced to exclude the possibility of natural horsesickness in this area. The whole outbreak was considered by the veterinary authorities to be something very exceptional, as they had never previously observed similar cases. There is the possibility, of course, of the disease not having been transmitted in the supposedly normal manner, i.e. by mosquitoes, in which case our contention of a possible biological alteration in the virus is supported. The time between the inoculation of the horses for immunization and the first death of one of the contact horses was 27 days. If we allow a period of three days for the appearance of the virus in the blood of the inoculated horses,

and a further period of 10 days for the interval between the infection of the contact horses by insects and their death, then there would remain a maximum of 14 days for the incubation period of the virus in the insect. This must be regarded, under our climatic conditions, with calm cool nights, as a relatively short incubation period in comparison with what we know about Yellow Fever and Dengue, i.e. if we accept at all the necessity for a certain period of development of the virus to render the insect infectious. The conditions for an insect transmission in this case must, therefore have been absolutely optimal. As an entomological survey was not successful, we do not know if this was the case. The transmission might possibly have been effected mechanically, but in any case the conditions under which the infection spread were apparently not normal, and it is, therefore, difficult to arrive at any definite conclusion as to the capacity of the strain developing normally in the insect host.

Our experiments were commenced in September, 1931. At that time we had only the O-virus strain at our disposal. We had hoped to obtain a normal field strain early in the season, but unfortunately one was not obtainable until towards the end of February, 1932.

In conducting the experiments discussed here it was our intention, firstly, to ascertain the most suitable time for feeding mosquitoes on infected horses. Secondly, to carry out some experiments in September or October with mosquitoes common in winter, i.e. before the commencement of the horsesickness season and the appearance of *Aëdes* species, and thirdly, to start preliminary experiments in November, when the first *Aëdes* mosquitoes generally appear, using those species most likely to be the natural vectors.

For these tests we used relatively small numbers of mosquitoes, having in mind the high infection index encountered in the Yellow Fever and Dengue work. Moreover, a positive result with a few specimens of a certain species would have been a more valuable indication than one with large numbers, as the method of injecting crushed mosquitoes was used. This method appeared to us to be the most suitable for preliminary work, for the reason that the virus being present in some part of the body in a virulent form, its presence would be revealed by injection of the whole mosquito even if it had not reached the salivary glands. The earliest interval chosen for injection was 5 days. The digestion of the blood in the intestines of the mosquitoes was usually completed after three days at the temperature we kept them, and there remained, therefore, no undigested blood to harbour the virus after five days. While blood was present in the intestines the presence of virus in emulsified mosquitoes would have been of no significance.

When all the above-mentioned experiments proved to be negative a further experiment was undertaken, using a large number of mosquitoes simultaneously. This concluded this series of tests.

A. Experimental Technique.

The mosquitoes used in these experiments were as a rule fed in the small cages described in the second paper, which were fastened to the skin of the horse with strips of plaster. Only in the last test

were they liberated in a mosquito-proof tent containing the infected horse. Except in the last experiment, the mosquitoes were fed during the night on horses kept in one of the ordinary stables.

During September and October, when working with *Culex*, the percentage of engorged specimens was reasonably high (up to 90 per cent.), without making any arrangements for increasing the humidity. Unsatisfactory results were only obtained with horses that were very sensitive to the mosquito bites. The feeding results with the *Aëdes* species were, on the whole satisfactory during November and December. In some cases, however, the percentage of engorged specimens was markedly less than in the experiments with *Culex*. Moreover a greater mortality was observed in the cages. Later it was found that the humidity was at fault, not being sufficiently high. The experiments with free mosquitoes in the fly-proof tent in the field, also yielded good results, without any precautions being taken. However, the night in question happened to be wet.

After feeding, the mosquitoes were kept in jam jars standing on wet cotton wool in larger glass jars, as has been described in the general section on technique. They were fed on 10 per cent. sugar water. This method proved satisfactory, although in some cases there was a considerable mortality.

In these experiments all mosquitoes were injected into normal horses not fed on them. They were first stunned by knocking the test tube containing them against the palm of the hand, after which they were crushed in normal horse serum in all cases, and injected subcutaneously into the neck of the horse. To commence with, ether was used to render them inert, and thus facilitate crushing, but its use was soon discontinued.

B. Mosquitoes.

In this group of experiments 807 infected mosquitoes were injected into susceptible horses. These mosquitoes included the following species:—

Culex theileri	254 specimens.
Anopheles squamosus	5 specimens.
Aëdes caballus	206 specimens.
Aëdes dentatus	18 specimens.
Aëdes hirsutus	145 specimens.
Aëdes lineatopennis	127 specimens.
Aëdes vittatus	52 specimens.

Whenever possible mosquitoes reared from larvae in the laboratory were used, and only when larvae were unobtainable had we to make use of adult specimens caught either in the field or in traps. The larvae of *Culex theileri* were very numerous in September and October, and could be caught in sufficient numbers amongst reeds growing near the banks of the Aapies River where the water was sluggish. Adults were very common during this period in the mosquito traps. Adults of *Anopheles squamosus* were only occasionally caught in the traps, and their larvae were rarely found in the Aapies River. The above-mentioned five species of *Aëdes* commenced to appear in November. At first they were relatively common, but

later on more difficult to find, and during the first half of January became comparatively rare. As there was very little hope of the climatic conditions improving, we artificially flooded the breeding places of *A. hirsutus*, *A. caballus* and *A. lineatopennis*, and were then able to catch these species in large numbers. The breeding place of *A. vittatus* had regularly been infested with dragon-fly larvae since January, and did not yield any further mosquitoes. Catching the dragon-fly larvae was not attended with success, as fresh eggs were continually being deposited. In the case of *A. hirsutus* and *A. vittatus* only specimens reared from larvae and pupae were used, whereas with *A. caballus* and *A. lineatopennis* adults caught on their breeding grounds or in the mosquito traps were also used in addition to bred specimens.

C. Virus Horses.

For this series of experiments we used a total of nine virus horses. By the term virus horse must be understood those infected horses used for feeding mosquitoes on. For simplifying later references, a short description of the course of the infection in each case will be given below, with an enumeration of the groups of mosquitoes fed on the respective horses.

Virus Horse 1 (*No*. 20181).—Injected on 7th September, 1931, intrajugularly with 5 c.c. O-virus (224th generation of 8th August, 1931).

Result: On 10th September p.m. first rise of temprature* up to 104°. The horse died 2½ days later. Fed *C. theileri*, group 1.

Virus Horse 2 (*No*. 20125).—Horse of experiment 1 (*Culex theileri* injection after ½ day). Fed *C. theileri*, group 2. For temperature see experiment 1.

Virus Horse 3 (*No*. 20183).—Injected intrajugularly on 21st September, 1931, with 5 c.c. O-virus (224th generation of 12th August, 1931).

Result: Temperature up to 23rd September a.m. normal, p.m. 102·5°; on 24th, 101·0° and 104 5°; on 25th, 103·4° and 105·0°; on 26th, 104·4° and 104·7°; and on 27th a.m., 105·0°. The horse died on the 27th p.m. Fed *C. theileri*, group 3.

Virus Horse 4 (*No*. 20261).—Injected on October 10th, with the same virus as the previous horse.

Result: Temperature normal up to 12th October a.m., p.m. 103·1°; on 13th, 103·3° and 106·1°; on 14th, 104·° and 106·1°; on 15th, 104·0° and 106·0°. The horse died during the following night. Fed *C. theileri*, groups 4 and 5, and *A. squamosus*, group 1.

Virus Horse 5 (*No*. 20196).—This horse had been used for experiment 3 (*C. theileri* injection after 16 days). It had also been infected some months earlier for other reasons with the Tzaneen strain of horsesickness. On 2nd November 5 c.c. O-virus (191st generation of 27th September, 1931) were injected intrajugularly.

Result: Temperature normal up to 9th November a.m., p.m. first rise up to 103·3°. Temperature on 10th, 102·4° and 104·0°; on 11th, 102·0° and 103·2°; on 12th, 102·0° and 103·9°; on 13th, 101·2° and 102·5°; on 14th, 101·3° and 102·0°. Thereafter the temperature was normal. The course of the disease was somewhat attenuated, and it is very likely that there was a certain amount of immunity against O-virus present, due to the earlier injections with T-virus. On this horse were fed *Aëdes caballus*, group 1; *A. dentatus*, group 1; and *Anopheles squamosus*, group 2.

Virus Horse 6 (*No*. 20184).—Used before in a negative experiment (experiment 2, *C. theileri*, 5 days). Injected 13th November with the same material of O-virus as virus horse 1.

* The temperatures of all experimental animals were taken twice daily; in the morning between 6.30 and 7, and in the afternoon between 3.30 and 4.

Result: Temperature normal up to 16th November a.m., p.m. rise to 104·4°. Temperature on 17th, 103·4° and 106·0°; on 18th a.m., 102·6°. The horse died the same afternoon. Fed *Aedes caballus*, group 2-3, and *A. lineatopennis*, group 1.

Virus Horse 7 (*No.* 20268).—Used for subinoculation in experiment 5 (*Culex theileri*, direct transmission). Injected on 23rd November with the same virus as the previous horse.

Result: Temperature normal up to 25th November a.m., p.m. 103·4°; on 26th, 102·8° and 106·0°; on 27th, 105·4° and 107·0°; and on 28th a.m., 106·5°. The horse died the same day. Fed *Aëdes caballus*, groups 4-6; *A. dentatus*, group 3; *A. hirsutus*, groups 2-3; and *A. lineatopennis*, group 2.

Virus Horse 8 (*No.* 20287).—Injected 5th December, 1931, with 5 c.c. O-virus (191st generation of 27th September).

Result: Temperature normal up to 7th December a.m., p.m. 103·6°; on 8th, 104·0° and 104·2° on 9th, 105·0° and 106·4°; and on 10th, 105·4° and 106·4°. The horse died during the following night. Fed *Aëdes caballus*, groups 7-10; *A. dentatus*, groups 4-5; *A. hirsutus*, group 4; *A. lineatopennis*, groups 4-7; and *A. vittatus*, group 1-2.

Virus Horse 9 (*No.* 20269).—Used previously for experiment 7 (*Aëdes caballus* injection after 5 days). Injected on 29th January intrajugularly with 5 c.c. O-virus (225th generation of 11th September, 1931).

Result: The temperature on 31st January a.m. was 102·0°; on 1st February, 103·4° and 106·1°; on 2nd February a.m., 105·1°. The same day the horse was killed for other purposes. Fed *Aëdes caballus*, group 11; *A. hirsutus*, group 5; and *A. lineatopennis*, group 8.

All these 9 cases developed the typical Dunkop form of horsesickness. Dikkop or mixed infections did not occur.

D. Experiments with *Culex theileri*.

To obtain an approximate idea of the amount of virus present in an infected horse during the fever reaction in connection with our mosquito work, we fed, as a preliminary experiment, a few specimens of *Culex theileri* (at that time other species were not at our disposal) on an infected horse shortly after the first rise of temperature. They were injected shortly afterwards (the following morning) into a normal horse. A series of experiments with several lots of mosquitoes fed at different times of the horsesickness reaction would certainly have been highly interesting, but on account of the expense we limited ourselves to this one test. With the same species four further experiments were made, using a total of 249 specimens. The interval varied between one minute and 25 days.

Virus: O-virus 192nd and 225th generations. Virus horses 1-4 and mosquito groups:—

Group 1.—Fed on virus horse 1 from 11th p.m. to 12th a.m. Second day of fever. Temperature 104·0°. 46 Specimens engorged (reared from larvae). Used for experiments 1-4.

Group 2.—Fed on virus horse 2 on 16th September p.m. Second day of fever. Temperature 105·4°. 18 Specimens engorged (reared from larvae). Used for experiments 3 and 4.

Group 3.—Fed on virus horse 3 from 25th September p.m. to the following morning. Second to third day of fever. Temperature 105·0°. About 100 specimens engorged (reared from larvae) used for experiments 2-4.

Group 4.—Some specimens fed on virus horse 4 on 13th and 14th October. First and second day of fever. Temperature 103·3° and 106·1°. Mosquitoes reared or caught. Used for experiment 5.

Group 5.—Fed on same horse from 13th October p.m. till the following morning. Second day of fever. Temperature 106·1°-104°. 135 Specimens engorged (reared from larvae). Used for experiments 2 and 4.

Experiment 1 (*H.S.* 1). 5 *Culex theileri*. *Injection*. *Interval* ½ *day*. *Horse* 20175.

On 12th September, 1931, 5 *C. theileri*, group 1, which had fed the night before on an infected horse, were injected subcutaneously.

Reaction: The temperature of the experimental horse on 15th September was 101·4° and 103·8°; the following day: 103·4° and 105·4°. The horse was very wild, and it was difficult to take the temperature regularly. The horse died on the night of 17th-18th September. The post-mortem showed typical horsesickness, dunkop.

The result of the experiment was thus *positive*.

Experiment 2 (*H.S.* 2). 50 *Culex theileri*. *Injection*. *Interval* 5 *days*. *Horse* 20184.

This horse was injected with 50 specimens of *C. theileri*, which had fed 5 days previously on an infected horse, viz., on 17th September with 10 specimens of group 1, on 1st October with 15 specimens of group 3, and on 19th October with 25 specimens of group 5. All the mosquitoes had fed on infected horses during the first or second day of fever.

Reaction: The temperature of horse 20184 remained normal, between 99° and 101°, up to 13th November, 25 days after the injection of the last batch of mosquitoes. Once the temperature rose to 102°, but only for half a day.

Immunity Test: The horse was subinoculated on 13th November with O-virus (224th generation of 17th August, 1931). It showed a typical horsesickness reaction and died 5 days after injection (*vide* virus horse 6).

The *result* of this experiment was thus *negative*.

Experiment 3 (*H.S.* 3). 50 *Culex theileri*. *Injection*. *Interval* 16 *days*. *Horse* 20196.

Into this horse 50 mosquitoes were injected which had been fed 16 days previously on an infected animal. On 28th September, 1931, 10 specimens of group 1 were injected, on 3rd October 15 of group 2, and on 12th October 25 specimens of group 3. These mosquitoes were fed on the infected horse during the second day of fever.

Reaction: The temperature of horse 20196 remained normal up to 2nd November, three weeks after the injection of the last group of mosquitoes, and during this time did not exceed 101·4°.

Immunity Test: On 2nd November the horse was injected intrajugularly with 5 c.c. of O-virus (191st generation of 27th September, 1931). Seven days later the temperature rose up to 103·3° F. and remained above 102·0° (afternoon temperatures) for five days. Thereafter it returned to normal. It was thus only a mild attack of horsesickness. The horse had been injected some time previous to this experiment with T-strain of horsesickness and had probably acquired a certain amount of immunity against O-virus. This is, however, rather exceptional.

Result: As there was no temperature reaction at all after the injection of the mosquitoes, we do not think that the result of the experiment is doubtful owing to the partial resistance of the animal against horsesickness. We consider we are justified, therefore, in regarding this experiment as *negative*.

Experiment 4 (*H.S.* 4). 140 *Culex theileri*. *Injection*. *Interval* 25 *days*. *Horse* 20259.

In this experiment 140 mosquitoes which had fed on infected horses 25 days before were used. On 7th October 14 specimens of group 1 were injected, on the 12th 2 of group 2, on the 21st 51 of group 3, and on 9th November 73 specimens of group 5. All these mosquitoes had been fed on virus horses during the first and second day of fever.

Reaction: The temperature of the horse did not exceed 101·2° up to 8th December, one month after the last injection of mosquitoes. It thus remained absolutely normal.

Immunity Test: On 15th February, 1932, the horse, whose temperature still remained normal, was injected with 5 c.c. blood of horse 20289 from experiment 16 (O-virus, 1st passage through mosquitoes). The fever reaction commenced the following day and the animal died 4 days later.

Result: Horse 20259 was thus susceptible and the experiment therefore *negative.*

Experiment 5 (H.S. 5). 9 Culex theileri. Direct transmission. Horse 20277.

On 13th October, 1931, 6 *Culex theileri* (group 4) were fed on virus horse 4. After they had taken up about half the normal amount of blood, the feeding was interrupted and the mosquitoes were immediately transferred to horse 20277 to complete their feeding on that animal. The interval was 85, 35, 40, 40, 60, and 50 seconds. The following day three more *C. theileri* of the same group were fed on both horses in the same manner. The interval was 95, 30, and 90 seconds.

Reaction: The temperature of the horse was normal for about three weeks, up to 5th November, when it rose to 102·5°. The maximum temperatures for the following days were: 101·2°, 101·0°, 101·9°, 99·3°, 101·6°, 104·1°, and 101·2°. During this period the horse showed muco-catarrhal discharges from both nostrils and an infection of one of the hind legs. The general appearance was very bad and it was killed on 12th November. The post-mortem was doubtful so far as horsesickness was concerned.

Subinoculation: 5 c.c. blood of this horse, taken on 7th November, was injected into horse 20268 on 11th November. The temperature of this second horse remained normal up to 23rd November; the maximum was 101·6°. On this day horse 20268 was injected with 5 c.c. O-virus (224th generation of 17th August, 1930). Two days later the temperature began to rise and the horse died 5 days later (*vide* virus horse 7).

Result: The observation period of the subinoculated horse, viz., 12 days, was certainly very short, and it is therefore not possible to give a definite statement about the result of this experiment. On the other hand, there is a strong indication that this experiment was also *negative.*

E. Experiments with *Anopheles squamosus.*

We were only able to make one test with a few specimens of *Anopheles squamosus*, a species which was exceptionally rare this season at Onderstepoort.

Virus: O-virus, 192nd and 225th generation. Virus horse 4 and 5 and mosquito groups:—

Group 1.—Fed on virus horse 4 on 15th October, third day of fever. Temperature 104·0°-106·0°. Three specimens engorged (caught as adults).

Group 2.—On 11th November two specimens (caught as adults) fed on virus horse 5. Third day of fever. Temperature 102·0°-103·2°.

Experiment 6 (H.S. 6). 5 Anopheles squamosus. Injection. Interval 5 days. Horse 20262.

On 20th October three specimens of *A. squamosus*, group 1, were injected into horse 20262, and on 17th November the two specimens of group 2. These mosquitoes had fed 5 days before the injection on infected horses.

Reaction: The temperature of the horse up to 17th December, one month after the injection of the last mosquitoes, varied between 98·5° and 101°, and was thus normal.

Immunity Test: On 4th March the horse was injected with blood from a natural case of horsesickness and died nine days later (*vide* virus horse 10, Losperfontein virus).

Result: The horse was thus susceptible and the experiment proved to be *negative.*

F. Experiments with *Aëdes caballus.*

Aëdes caballus, as we have stated before, is one of the common species of *Aëdes* round Onderstepoort, and in the later stages of the work it was quite regularly obtained for experiments.

Two experiments were undertaken, using a total of 122 specimens at intervals of 5 and 15-16 days.

Virus: O-virus, 192nd and 225th generation. Virus horses 5, 6, 7, and 8, and mosquito groups:—

Group 1.—Fed on virus horse 5 on 11th November p.m. till the following morning. Third day of fever. Temperature 103·2°-102·. Twenty specimens engorged (hatched from pupae). Used for experiment 7.

Group 2.—Fed on virus horse 6 during the night, 17th-18th November. Second day of fever. Temperature 106·0°-102·6°. Six specimens engorged (reared from larvae). Used for experiment 7.

Group 3.—Fed on the same virus horse on 18th November a.m. Second day of fever. Temperature 102·6°. One specimen engorged (reared from larva). Used for experiment 7.

Group 4.—Fed on virus horse 7 on 27th November p.m. First to second day of fever. Temperature 102·8°-106·0°. Eighty specimens engorged (hatched from pupae and caught as adults). Used for experiments 7 and 8.

Group 5.—Fed on the same virus horse during the following night. Second day of fever. Temperature 106·0°-105·4°. Twenty-eight specimens engorged (hatched from pupae and caught as adults). Used for experiment 8.

Group 6.—Fed on the same virus horse on 27th November. Third day of fever. Temperature 105·4° and 107·0°. Thirty specimens engorged (caught as adults). Used for experiment 8.

Group 7.—Fed on virus horse 8 on 7th December p.m. First day of fever. Temperature 103·6°. Twelve specimens engorged (caught as adults). Used for experiment 7.

Group 8.—Fed on the same virus horse on 8th December p.m. Second day of fever. Temperature 104·2°. Twenty specimens engorged (caught as adults). Used for experiment 7.

Group 10.—Fed on the same virus horse one day later. Third day of fever. Temperature 105·0°-106·4°. Twenty-five specimens engorged (caught as adults). Used for experiment 7.

Experiment 7 (H.S. 7). 94 Aedes caballus. Injection. Interval 5 days. Horse 20269.

In this experiment ninety-four specimens of *A. caballus*, which had fed about five days previously on experimentally infected horses, were injected in all. On 17th November ten specimens of group 1 were injected, on the 23rd six of group 2 and one of group 3, on 1st December thirty-three of group 4, on the 12th ten of group 7, on the 14th eleven of group 8 and twenty-three of group 10. The mosquitoes had fed on the virus horses during the first to third days of fever, except group 1, which had fed on the first or second day.

Reaction: The temperature showed a few slight rises after the injection of the mosquitoes, but otherwise remained within the normal limits during an observation period of 1½ months.

After the first injection, a slight rise up to 101·6° on the fourth day occurred. The second injection was followed by a rise up to 101·3° one day later. After the third injection of thirty-three specimens the temperature went up to 104·0° on the day of injection, but fell the next day down to 101·0°, and did not exceed 101·2° during the following ten days. The last two injections of 44 specimens followed one another directly. The temperature rose up to 102·0° a few days later, remained on the same level the next day, and then dropped again to normal. Except for these fluctuations, the temperature remained between 99·3° and 101·2° up to 28th January, 1932.

Immunity Test: On 29th January, 1932, the horse was injected with 5 c.c. O-virus (225th generation of 11th September, 1930). The first signs of fever began to develop two days later. The horse was killed four days p.i. and the post-mortem showed typical signs of the dunkop form of horsesickness (*vide* virus horse 9).

Result: The horse was thus susceptible to horsesickness and the experiment has to be looked upon as *negative*. The temperature reactions were not high enough to indicate even a very milk attack of horsesickness.

Experiment 8 (*H.S.* 13). *Aëdes caballus. Injection. Interval* 15-16 *days. Horse* 20291.

This horse was injected with twenty-eight specimens of *A. caballus* which had fed 15 or 16 days before on an infected horse. On 12th December, 1931, ten specimens of group 4, five of group 5, and thirteen of group 6 were injected. These mosquitoes had fed on a virus horse during the end of the first until the beginning of the third day of fever.

Result: The temperature of the horse remained normal, varying during the first month following the injection between 98·4° and 101·4°. The animal was kept under observation for a further 70 days. Between 26th January and 10th March, the temperature was somewhat irregular, rising on six days to above 102°, with 104·8° as maximum. On one occasion only did the temperature remain above 102° for two consecutive days, otherwise elevated temperatures were not maintained for longer than half a day at a time. On 23rd March, 1932, the horse was used in experiment 32 (Eshowe virus, *A. dentatus*, fed, 15 days and longer). No definite febrile reaction resulted.

Immunity Test: On 3rd June, 1 c.c. Eshowe virus was injected subcutaneously into this horse. The temperature commenced rising five days later and the horse died from dunkop four days later.

Result: The immunity test showed that the horse was normally susceptible to Eshowe virus. There is every reason to believe that it would have been susceptible to O-virus, as well as have been all horses tested from the same lot. As no typical fever reaction followed the injection of mosquitoes, the experiment has to be regarded as *negative*.

G. Experiments with *Aëdes lineatopennis*.

Two experiments, using 33 specimens, were carried out with this species.

Virus: O-virus (192nd and 225th generations). Virus horses 6, 7, and 8, and mosquito groups:—

Group 1.—Five specimens (reared from larvae) fed on virus horse 6 on 17th November, 1931. Second day of fever. Temperature 106°-102.6°. Used for experiment 9.

Group 2.—Thirteen specimens (reared from larvae) fed on virus horse 7 on 26th November. Second day of fever. Temperature 106°-105·4°. Used for experiment 10.

Group 3.—Thirty-three specimens (caught as adults) fed on virus horse 7 on 27th November. Third day of fever. Temperature 105·4°-107°. Used for experiment 10.

Group 4.—Three specimens (caught as adults) fed on virus horse 8 on 7th December. First day of fever. Temperature 103·6°. Used for experiment 10.

Group 5.—Five specimens (caught as adults) fed on virus horse 8 on 8th December. Second day of fever. Temperature 104·2°. Used for experiment 10.

Group 7.—One specimen (caught as adult) fed on same virus horse on 9th December. Third day of fever. Temperature 105°-106·4°. Used for experiment 10.

Experiment 9 (*H.S.* 8). 5 *Aëdes lineatopennis. Injection. Interval* 5 *days. Horse* 20270.

This horse was injected on 23rd November, 1931, with 5 *Aëdes lineatopennis* (group 1), which had fed about 5 days previously on an infected horse during the second day of fever.

Result: The temperature of the horse remained normal (98·4°-101·4°) up to 1st December, when it was injected with *A. hirsutus* (fed on a horse, O-virus, 5 days previously, experiment 11; result: negative). On 20th February, 1932, the animal was injected with *Aëdes* spp. (which had fed on a horse, O-virus after mosquito passage, 7-9 days previously, experiment 11; result: negative).

Immunity Test: On 19th March the horse was injected with Eshowe virus (1st generation), and died of horsesickness 5-6 days later.

Result: The experiment was therefore *negative*, as the horse proved to be susceptible.

Experiment 10 (*H.S.* 12). 28 *Aedes lineatapennis. Injection. Interval* 5 *days. Horse* 20288.

Twenty *A. lineatopennis* (eleven of group 2 and nine of group 3) were injected into the horse on 12th December, and two days later three of group 4, four of group 5, and one of group 7.

Reaction: The temperature of the horse remained normal (99°-101°) for nearly three months, except for short rises directly after the injections of the mosquitoes, when the temperature reached 102·1° one afternoon.

On 9th March the horse was used for experiment 24, and was injected with *A. caballus* and *A. lineatopennis* which had fed on a horse (Eshowe virus) 7 days previously. This experiment was positive and proved that the horse was susceptible to horsesickness.

Result: The experiment was therefore *negative.*

H. Experiments with *Aëdes hirsutus.*

It was only possible to carry out one test, using 30 specimens, with this species.

Virus: O-virus (192nd and 225th generations). Virus horses 7 and 8, and mosquito groups:—

Group 2.—Seventeen specimens (reared from larvae) fed on virus horse 7 on 26th November, 1931, p.m. First and second day of fever. Temperature 102·8-106°. Used for experiment 11.

Group 3.—Ten specimens (reared from larvae) fed on the same virus horse from 26th November p.m. until the following morning. Second day of fever. Temperature 106°-105·4°. Used for experiment 11.

Group 4.—Fifteen specimens (reared from larvae) fed on virus horse 8 on December 9th. Third day of fever. Temperature 105°-106·4°. Used for experiment 11.

Experiment 11 (*H.S.* 10). 30 *Aëdes hirsutus. Injection. Interval* 5 *days. Horse* 20270.

Fifteen specimens (group 2) were injected into the horse on 1st December, 1931, five of group 3 the following day, and ten (group 4) on 14th December. They had fed on the virus horses during the first to third days of fever.

Reaction: The temperature varied between 97·4° and 101·6° up to 19th February, more than a month after the last injection. It thus remained normal. On 20th February, 1932, the horse was injected, in experiment 17, with 17 *Aëdes* spp. which had fed on a horse (O-virus after mosquito passage) 7-8 days previously. There was no marked temperature reaction during that month.

Immunity Test: On 19th March the horse was injected intrajugularly with E-virus (1st generation). Three days later the temperature began to rise, and the horse died of horsesickness 5-6 days later.

Result: The horse thus proved to be susceptible, and the experiment must be regarded as negative.

I. Experiments with *Aëdes dentatus.*

Two experiments, using 18 specimens, were carried out with this species.

Virus: O-virus (192nd and 225th generation). Virus horse 7 and 8, and mosquito groups:—

Group 3.—Thirteen specimens (caught as adults) fed on virus horse 7 on 26th November p.m. First to second day of fever. Temperature 102·8°-106°. Used for experiment 12.

Group 4.—Three specimens (reared from larvae) fed on virus horse 8 on 7th December p.m. First day of fever. Temperature 103·6°. Used for experiment 13.

Group 5.—Twelve specimens (reared from larvae) fed on virus horse 8 on 9th December. Third day of fever. Temperature 105°-106·4°. Used for experiment 13.

Experiment 12 (*H.S.* 11). 6 *Aëdes dentatus*. *Injection*. *Interval* 5 *days*. *Horse* 20271.

This horse was injected on 2nd December, 1931, with 6 *A. dentatus* (group 3) which had fed 5 days previously on an infected horse.

Reaction: The temperature of the horse remained normal during the first 11 days p.i., showing a maximum of 101·4°. On 14th December the temperature rose to 103° and on the following seven days was: 100·4° and 102·3°, 101·3° (a.m.), 101 and 102°, 102° (a.m.), 98 (a.m.), 98° (a.m.), and 103° (a.m.). During this reaction the horse showed discharges from the nostrils and swelling of the glands, due to an attack of rhinitis. On 23rd December the temperature was again normal and remained below 101° for more than two months, i.e. up to 26th February, 1932.

On 27th February the horse was injected with *Aëdes* spp. which had fed on a horse (O-virus after mosquito passage) 8-9 days previously (experiment 18). During an observation period of 40 days no reaction resulted. On 8th April the horse was used as a control in experiment 31 (Eshowe virus, *A. hirsutus*, feeding). No temperature reaction occurred during a period of more than a month.

The result of this experiment must be regarded as negative. We did not consider it necessary to test this animal for immunity, as all animals from the same source, so far as they were tested, proved to be susceptible.

Experiment 13 (*H.S.* 9). 12 *Aëdes dentatus*. *Injection*. *Interval* 16 *days*. *Horse* 20280.

Three specimens (group 4) were injected into this horse on 23rd December, 1931, and 9 (group 5) on the following day. The mosquitoes had fed about 16 days previously on an infected horse during the first and third days of the fever reaction.

Reaction: The horse did not show any fever reaction during the month following the last injection of the mosquitoes. The temperature remained below 101°, except on a few separate days. The highest temperature registered was 101·8°. The horse was kept under observation for a further 44 days up to 8th March. Three times during this period the temperature surpassed 102°, but only half a day in each case. The maximum temperature was 102·5°. On 9th March the horse was used for experiment 30 (Eshowe virus, *A. hirsutus*, injection). No temperature reaction occurred in this experiment either.

Immunity Test: The horse was injected with Eshowe virus on 27th May. Four days later the temperature commenced rising, and on the 8th day p.i. the horse died from dunkop horsesickness.

Result: The horse did not show any temperature after the injection of mosquitoes, but later proved to be susceptible to another strain of horsesickness. For reasons already stated in other experiments, we may regard the horse as susceptible to O-virus, and the experiment was therefore *negative*.

J. Experiments with *Aëdes vittatus*.

Two experiments, using 52 specimens, were carried out with this species.

Virus: O-virus (192nd generation). Virus horse 8, and mosquito groups:—

Group 1.—Thirty-one specimens (reared from larvae) fed on virus horse 8 on 7th December, 1931. First day of fever. Temperature 103·6° used for experiments 14 and 15.

Group 2.—Forty specimens (reared from larvae) fed on the same horse on 9th December. Third day of fever. Temperature 105°-106·4°. Used for experiments 14 and 15.

Experiment 14 (*H.S.* 14). 20 *Aëdes vittatus*. *Injection*. *Interval* 5 *days*. *Horse* 20289.

Ten specimens (group 1) were injected into the horse on 2nd December, and two days later ten more of group 2. The mosquitoes had fed on an infected horse during the first and third days of fever.

Reaction: The temperature remained normal (98·2°-101·6°) up to 8th February, 1932, nearly two months after the last injection of the mosquitoes, except on one occasion, about a month after the injections, when it rose to 103·4°, but dropped again the next day to 99·3°.

Immunity Test: On 8th February, 1932, the horse was used for experiment 16,, and was injected with a large number of *Aëdes* spp., 6 days after they had fed on an infected horse. This experiment was positive and proved the susceptibility of the horse for horsesickness.

Result: The experiment must, therefore, be regarded as *negative*.

Experiment 15 (*H.S.* 15). 32 *Aëdes vittatus. Injection. Interval* 15 *days. Horse* 20297.

Fifteen specimens (group 1) were injected into the horse on 23rd December and 17 (group 2) on the following day. These mosquitoes had fed on a virus horse approximately 15 days before during the first and second days of fever.

Reaction: The temperature of the horse remained normal, the highest temperature registered during the first month after the injection being 101°. The horse was kept under observation for almost a further two months, and during this period the temperature only reached 102° for half a day.

On 23rd March the horse was used for experiment 19 (Losperfontein virus, *A. caballus*, feeding). In this experiment also no fever reaction occurred.

Immunity Test: On 11th May, 1932, the horse was injected with 5 c.c. Losperfontein virus (from original case). After an incubation period of 7 days the temperature commenced to rise. The horse died 4 days later, the maximum temperature being 105°.

Result: The horse was thus susceptible to the Losperfontein strain of horsesickness and would very likely have reacted equally well to O-virus. The experiment has therefore to be regarded as *negative*.

K. Experiments with Larger Numbers of Different *Aëdes* Species.

In the last experiment of this series we injected a larger number of specimens of the three most important species of mosquitoes, *Aëdes caballus*, *A. hirsutus* and *A. lineatopennis* simultaneously, hoping to obtain a positive result in this manner. The mosquitoes were injected one week after their having fed on the infected horse, thus giving the virus a certain time in which to multiply or develop.

Virus: 225th generation of O-virus. Virus horse 8, and mosquitoes:—

Aëdes caballus, group 11. On 1st February a large number of newly hatched mosquitoes were brought into contact with virus horse 9 in a mosquito-proof tent, standing in the veld near the laboratories. Second to third day of fever. Temperature 106·1°-105·1°. The engorged mosquitoes were caught the following morning and kept in the warm room.

A. lineatopennis, group 6. Mosquitoes from the same source as *A. caballus*, group 11, and treated in the same manner.

A. hirsutus, group 5. A large number of hatched adults fed on the same virus horse at the same time as the other two groups.

Experiment 16 (*H.S.* 16). 294 *Aëdes spp. Injection. Interval* 6 *days. Horse* 20289.

The horse in this experiment had been used previously in experiment 14 *Aëdes vittatus*, injection after 5 days), but it had not shown any temperature reaction during an observation period of 56 days. It was in poor condition and showed filling of the hind legs.

On 8th February, 1932, the horse was injected subcutaneously with 294 mosquitoes. 85 *Aëdes caballus* (group 11), 115 *A. hirsutus* (group 5), and 94 *A. lineatopennis* (group 8). These mosquitoes had fed on the night of 1st to 2nd February, thus about 6½ days previously, during the first to third day of fever. The injections were made on both sides of the neck, and marked swellings developed at the sites of injection, no doubt due to the very large number of mosquitoes used, which naturally were not sterile.

Reaction (see fig. 2): The temperature during the week preceding the injection was 99·0°-101·4°; on the day of injection 99° and 100·6°. The following day the temperature rose to 104·0° in the morning, in the afternoon to 104·4°, and on 10th February, the second day after injection, 102·0° and 103·0°, and on the 11th (third day a.m.) 101·6° was registered. The swellings at the sites of injection were still more marked the day after the injection, but a slight reduction was noticeable on the second day, followed, however, by further enlargement on the third day. The high rise of temperature shortly after the injections was undoubtedly due to a local reaction set up by the injected mosquitoes. When the swellings decreased the temperature also went down.

On 11th February p.m. (third day) the temperature rose from 101·6° up to 104·0°, reaching, on the 12th (fourth day) 105·4° and 105·8°, and on the 13th a.m. (fifth day) 105·3°. During the afternoon of the same day the temperature came down to 102·5°, and the horse died during the following night. The post-mortem was unfortunately not altogether clear cut as to the cause of death, owing to the advanced state of decomposition of the carcass.

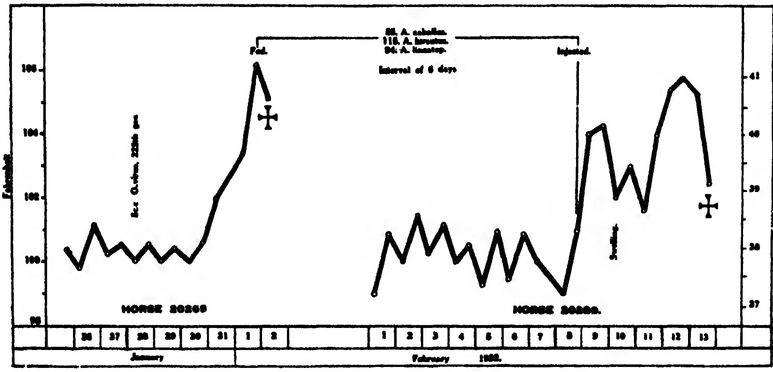

Fig. 2.

According to the course the reaction took, the indications pointed strongly to horsesickness being the cause of death. In order to establish this fact, another animal was subinoculated with blood from this horse.

Subinoculation: On 15th February horse 20259 was injected intrajugularly with 5 c.c. blood of horse 20289, bled on 12th February.

This new horse had been used before in experiment 4 (*Culex theileri*, injection after 25 days). During an observation period of more than 3 months it had not shown any significant rise in temperature. The temperature during the last week before the injection was 98·8° to 101·1°.

On the day after injection, 16th February, the temperature was 100·0° in the morning, but rose in the afternoon up to 104·5°. On the second day it was 105·0° and 105·4°, on the third day 105·7° and 106·6°, and on the fourth day 106·3° and 105·4°. The horse died during the night. The post-mortem showed the typical lesions of the dunkop form of horsesickness.

The very short incubation period of only 1½ days in this case was remarkable.

The *result* of the main experiment was thus *positive* as confirmed by subinoculation of blood.

L. Results of the Experiments with Pure O-virus.

In the first preliminary experiment a positive result was obtained by the injection of five mosquitoes which had fed the night before on an infected horse 24 hours after the first rise of temperature, during a horsesickness reaction. At that time, therefore, the concentration of virus was so high, that it must have been taken up by at least one of the five mosquitoes. To determine exactly the

optimum range for transmission experiments more tests of this kind would have been of value, but they seemed to us not to be absolutely justified considering the costs involved. We considered ourselves as safe, by analogy with the results obtained in the Yellow Fever and Dengue work, by commencing the experiments after the first rise of temperature above 102·5° and finishing them after the third day of fever.

In these experiments, as in all the others of this series, the mosquitoes were crushed and injected subcutaneously into susceptible horses, not fed on them. We thought that this method would give better and quicker results in a preliminary investigation into the actual transmitters. A negative result would indicate that there was no virus present in any part of the insect's body, but a positive test naturally would need confirmation through feeding experiments.

As a rule we began with the injection of the mosquitoes 5 days after their feeding on an infected horse. Normally all the blood in the intestines of a fully engorged mosquito was digested after 2-3 days at the temperature they were kept at in the warm room.

The experiments with *Culex theileri*, which were carried out in September and October, when no other mosquitoes could be obtained, were planned mainly to develop the experimental technique. This species is as common in winter as in summer, and does not depend on rain as it breeds in permanent water. From an epidemiological point of view, therefore, it does not fit in with the transmission at all.

All experiments with *C. theileri* were negative. The number of specimens used was certainly sufficient. After five days 50 specimens were injected, after 16 days 50 and after 25 days 140, altogether 240 specimens. We therefore considered that *C. theileri* could be excluded from the experimental point of view as a transmitter of horsesickness. However, as will be seen later, this conclusion is not absolutely justified.

In the further experiments 5 species of *Aëdes* were used, viz., *A. caballus*, *A. dentatus*, *A. hirsutus*, *A. lineatopennis* and *A. vittatus*. The entomological survey, undertaken under adverse climatic conditions, had indicated that these species had to be regarded as potential transmitters.

The results of the first set of experiments were certainly unexpected, all of them, as it happened, being negative. We used the following numbers of mosquitoes:—

A. caballus interval, 5 days, 94 specimens; 15 days, 28 specimens.

A. dentatus, interval 5 days, 6 specimens; 15 days, 12 specimens.

A. hirsutus, interval 5 days, 30 specimens.

A. lineatopennis, interval 5 days, 33 specimens.

A. vittatus, interval 5 days, 20 specimens; 15 days, 32 specimens.

Of these 5 species, 183 specimens were injected after 5 days and 72 after 15 days. This number is certainly not insignificant when we compare it with the high percentage of infections in Yellow Fever and Dengue.

What were the reasons for the negative results? Naturally, the first possibility to be considered is that the species used were not the natural transmitters. We have already laid stress upon the fact that the entomological survey pointed clearly to these species as the potential transmitters. Further, almost daily observations in the field after these unexpected results could not give us any other indication of importance.

Another possibility, already mentioned above was, that either the virus had lost its developmental capacity in mosquitoes, or that this was at least reduced to a certain extent. We used the 192nd and 225th generations of the O-virus strain which had been isolated more than 30 years previously from a natural case of horsesickness. It was, therefore, reasonable to suppose that the strain, never having been in contact with mosquitoes for such a long time, would have lost its normal capacity of developing in mosquitoes.

In considering the following facts the results are doubly strange. In contrast to the virus of yellow fever and dengue, the horsesickness virus, in vitro, is very resistant. It remains virulent in blood at ordinary room temperature for years and even in a putrefactive state its virulence is not easily reduced. In the mosquitoes, however, its virulence was completely destroyed after 5 days. If we had any reason to doubt the results of our entomological survey, we would have given up the further work with these *Aëdes* species, regarding them either as non-transmitters or in any case not as transmitters of importance.

Undoubtedly the best thing we could do was to abandon the O-virus and take another strain derived from a fresh case of horsesickness. Every possible effort was, however, made in this direction for more than a month, without success. The result was merely a loss of valuable time.

We decided thereupon to make at least one more effort with the O-virus strain, this time injecting simultaneously a large number of mosquitoes into one horse after an interval of 6-7 days. Supposing that the normal developmental capacity of the virus in the mosquitoes was diminished, there would be a better chance of getting positive results when using a large number of insects. In fact, this experiment proved positive without any marked lengthening of the incubation period. We injected 85 *A. caballus*, 115 *A. hirsutus* and 94 *A. lineatopennis*, altogether 294 specimens, or nearly 40 specimens more than in all the previous experiments together. It was thus possible to keep the virus virulent in the bodies of mosquitoes for at least 6-7 days. We had hoped that this short passage of the strain would enable us to carry on with the work. In the following chapter the results of the further experiments with this strain will be described.

VI. EXPERIMENTS WITH O-VIRUS AFTER ONE PASSAGE THROUGH MOSQUITOES.

In the preceding chapter we have recorded one positive experiment obtained by the injection of about 300 mosquitoes belonging to three different species (experiment 16). The virus had remained in the mosquitoes for about 6-7 days. We hoped that this would constitute a beginning for the adaptation of the virus to live in the mosquito transmitters, and that by a series of similar short passages through mosquitoes the virus would regain its normal developmental capacity in these insects. We still believed that we were right in our choice of the natural invertebrate host of the virus.

As the results were not satisfactory, we conducted two experiments.

A. Mosquitoes: Experimental Technique and Virus Horses.

Mosquitoes.—For these experiments we chose mainly three species of *Aëdes*, viz., *A. caballus*, *A. hirsutus* and *A. lineatopennis*. These we regarded as the most probable transmitters of horsesickness, on grounds stated in detail in one of the preceding chapters.

In the two experiments together we injected the following mosquitoes:—

Aëdes caballus, 185 specimens; *A. lineatopennis*, 121 specimens.
A. dentatus, 11 specimens; *Aëdes* spp., 3 specimens.
A. hirsutus, 37 specimens.

Most of these mosquitoes were reared from larvae; only a small percentage (31 specimens) were caught as adults.

Experimental Technique.—In the first experiment the mosquitoes were brought into contact with the infected horse in the same mosquito-proof tent we used in experiment 16 of the preceding chapter. Certainly, more than 1,000 specimens were liberated in this tent, but only 95 engorged themselves on the horse. A very large number died without taking any food. It was a dry day and night, and we are convinced that the low humidity was the reason for the bad results. We therefore never used this tent again.

For the next experiment we built another tent (described in the paper on technique) near one of the buildings of the laboratory, and during the feeding of the mosquitoes we had water running down from the roof to keep the air inside the tent sufficiently moist. The mosquitoes were again liberated in the tent, and the result was much better. Nearly 350 mosquitoes engorged themselves on the horse.

The engorged mosquitoes of the first experiment were kept in the warm room in small jars, those of the second partly in jars and partly in special cages surrounded by wet hessian. As virus horses we used horse 20289 from experiment 16 and horse 20259 which had been injected with blood of the preceding horse.

B. Experiments.

Experiment 17 (*H.S.* 17). 57 *Aëdes spp. Injection. Interval* 7-8 *days. Horse* 20270.

The mosquitoes used in this experiment were fed on horse 20289 of experiment 16, which had reacted after an injection of crushed mosquitoes belonging to three different *Aëdes* spp.

Mosquitoes: A. caballus, group 12. Fed on horse 20289 on 12th February. Five specimens engorged. First to second day of fever. Temperature 105·4°-105·8°. Mosquitoes caught as adults.

A. caballus, group 13. Fed on the same horse between 12th February p.m. and the next morning. Twenty-seven specimens engorged. Second day of fever. Temperature 105·8°-105·3°. Mosquitoes reared from larvae.

A. lineatopennis, group 9. Fed on the same horse during the night of 11th to 12th February. First day of fever. Temperature 104°-105·4°. Forty-one specimens engorged. Mosquito caught as adults.

A. hirsutus, group 6. Fed on the horse, together with *A. caballus*, group 13. Two specimens, reared from larvae, engorged.

The horse in this experiment had been used before in experiment 11 (*Aëdes hirsutus*, injected after 5 days). During more than two months after the last injection of mosquitoes the temperature of the horse had remained normal (between 97·4° and 101·6°), and the result of the test had been negative.

On 20th February, 1932, the horse was injected with 4 *A. caballus*, group 12, twenty-four of group 13, with 2 *A. hirsutus* of group 6, and 27 *A. lineatopennis* of group 9, altogether with fifty-seven specimens. The mosquitoes had fed 7-8 days before on the infected horse during the first and second day of fever. There was a swelling at the site of injection, which decreased after a few days and disappeared totally later on.

Reaction: The day after the injection the temperature rose up to 102·6°, but fell the following morning to 99·6° and did not surpass 101·7° during the next 5 days. The horse was kept under observation for 28 days. During this period the temperature was never higher than 101·5°, ordinarily remaining below 100·5°. There was therefore no temperature reaction.

Immunity Test: On 19th March the horse was injected intrajugularly with 5 c.c. E-virus, 2nd generation (see virus horse 12). Three days later the temperature began to rise and after two more days the horse died.

Result: The horse died of horsesickness, and the experiment proved to be negative. The immunity test was actually made with another strain of virus, but nevertheless the horse may be regarded as having been susceptible to O-virus also, as all immunity tests carried out on other horses of the same lot of animals were successful.

Experiment 18 (*H.S.* 18). 300 *Aëdes spp. Injection. Interval* 8-9 *days. Horse* 20271.

The virus horse used for feeding the mosquitoes on was horse 20259 of experiment 16. It was inoculated with blood from horse 20289, which reacted after an injection of nearly 300 mosquitoes previously fed on a horse infected with O-virus.

The mosquitoes fed on this horse belonged to six different species, *A. caballus*, *A. dentatus*, *A. hirsutus*, *A. lineatopennis*, the common mosquitoes used regularly in these experiments, and *A. cumminsi* and *A. punctothoracis*, two species which are usually rare at Onderstepoort.

Mosquitoes: A. caballus, group 14. Fed on horse 20259 between 17th February and the next morning. Second day of fever. Temperature 105·4°-105·7°. Forty-three specimens (reared from larvae) engorged.

A. caballus, group 15. Fed on the same horse the following night. Third day of fever. Temperature 106·6°-106·3°. 133 Specimens (reared from larvae) engorged.

A. cumminsi, group 1. Fed on the same horse, together with *A. caballus*, group 14. One specimen engorged (reared from larva).

A. dentatus, group 7. Fed on the same horse, together with *A. caballus*, group 14. Eleven specimens engorged (reared from larvae).

A. hirsutus, group 7. Fed on the same horse, together with *A. caballus*, group 14. Seventeen specimens engorged (reared from larvae).

A. hirsutus, group 8. Fed on the same horse, together with *A. caballus*, group 15. Nineteen specimens engorged (reared from larvae).

A. lineatopennis, group 11. Fed on the same horse, together with *A. caballus*, group 14. Twenty-seven specimens engorged (reared from larvae).

A. lineatopennis, group 12. Fed on horse, together with *A. caballus*, group 15. Ninety-one specimens engorged (reared from larvae).

A. punctothoracis, groups 1 and 2. Group 1 fed with *A. caballus*, group 14; group 2 with 15. Two specimens engorged (reared in the laboratory).

The horse for this test had been used before in experiment 12 (*Aedes dentatus*, injection after 5 days). 12-19 days after the injection of the mosquito it had shown an irregular temperature reaction accompanied by discharges from the nostrils, due very probably to an ordinary rhinitis. It was kept under observation for nearly 3 months (87 days). The temperature only passed the 101° limit for one afternoon. The experiment had, therefore, to be regarded as negative.

On 27th February, 1932, the horse was injected subcutaneously with the following 300 mosquitoes:—

157 *A. caballus:* 31 of group 14, 126 of group 15.
1 *A. cumminsi.*
11 *A. dentatus*, group 7.
35 *A. hirsutus:* 16 of group 7, 19 of group 8.
94 *A. lineatopennis:* 22 of group 11, 72 of group 12.
2 *A. punctothoracis:* 1 of group 1, 1 of group 2.

These mosquitoes had fed on the infected horse 20259 on the second and third day of fever between 17th February p.m. and 19th a.m., thus 8-9 days previously.

Reaction: The day after the injection of the mosquitoes the temperature rose up to 103·2° in the morning, but fell to 101·2° in the afternoon. It was 101° the next morning, and rose again in the afternoon to 102·8°. The following day the temperature was nearly normal (100°-100·8°). The horse was kept under observation for 40 days, but no fever reaction followed, the temperature remaining between 98·3° and 101·8°, exceeding 101° only once for half a day.

On 8th April the horse was used for experiment 31 (Eshowe-virus, *Aedes hirsutus*, feeding, subinoculation). During an observation period of more than one month 101° was the highest temperature recorded.

Result: As no fever reaction resulted after the injection of the mosquitoes, the experiment has to be regarded as *negative*. An immunity test was not carried out on this horse. It could be omitted, as quite a number of horses of the same origin always proved to be susceptible when tested.

C. Results of Experiments.

In the experiments described in the foregoing section we had our positive result after injection of a large number of mosquitoes belonging to different species of *Aëdes* which had been injected about 6 days after their having fed on a horse experimentally infected with O-virus. On this horse and on other animals which had been injected with blood of the previous horse, we fed a large number of *Aëdes* mosquitoes.

In two experiments these mosquitoes were injected after an interval of 7-8 and 8-9 days into two susceptible horses. Altogether we used 357 specimens, viz., 185 *A. caballus*, 121 *A. lineatopennis*, 37 *A. hirsutus*, 11 *A. dentatus*, 2 *A. punctothoracis* and 1 *A. cumminsi*. According to our mosquito survey the first three species had to be regarded as very promising possible transmitters. Both experiments were negative, proving that after a maximum interval of 9 days there was no virulent virus left in any of the mosquitoes.

Our hope, that through a relatively short sojourn in mosquitoes, the virus would regain at least part of its normal developmental capacity, was not fulfilled. It is possible that, if still larger numbers of mosquitoes were used, positive results could be obtained, but the chances of modifying the virus successfully by this method do not seem to be very great. We therefore ended these attempts, as we had obtained two new strains of horsesickness in the meantime which seemed to be suitable for our experiments.

VII. EXPERIMENTS WITH LOSPERFONTEIN VIRUS.

At the commencement of March, 1932, we obtained fresh material from a case of horsesickness which had occurred in a mule at Losperfontein, near Brits (Transvaal), it being a typical case of the "Dikkop" form of the disease. We were unable to trace the history of this case with certainty, but it was ascertained that most or all of the mules at Losperfontein, which is a Government Irrigation Settlement, had been immunized against hirsesickness in December, 1931, by means of the Onderstepoort O-virus strain. A certain number of deaths had occurred following upon the immunization and throughout February, 1932, a few further horsesickness deaths were encountered, of which the above-mentioned mule formed one, from which our material had been obtained. As certain indications pointed to this being a naturally contracted case of horsesickness, we injected one of our horses with this material and used it for feeding mosquitoes on. Except for a somewhat longer incubation eriod the course of the disease closely resembled a typical O-virus infection.

For reasons outlined below, we did not regard this strain as very suitable for our purpose and, therefore, injected only one horse with it.

A. Virus Horse.

The following horse was injected with this strain for the purpose of feeding mosquitoes:—

Virus horse 10 (=horse 20262). Injected on 4th March, 1932, intrajugularly with 5 c.c. blood taken the previous day from a mule at Losperfontein, showing typical symptoms of dikkop.

[This virus horse had been used in October, 1931, for experiment 6 (O-virus, *Anopheles squamosus*, injection), but had not shown any fever reaction.]

After injection the temperature remained normal for the four days up to 8th March. On 9th March the temperature was 100·2° (a.m.) and 103·7° (p.m.), the 10th 102·5° and 103·0°, the 11th 104·6° and 106·3°, the 12th 105·3° and 106·6°, and on the 13th 106·0° (a.m.). The same day the horse died, the post-mortem showing typical symptoms of dunkop.

B. Mosquitoes and Experimental Technique.

Mosquitoes.—At the beginning of March, 1932, when the experiments with the Losperfontein strain were being conducted, *Aëdes caballus* was present in large numbers. They were hatching out in the laboratory from larvae caught in the field and were also present as adults in their breeding grounds. *Aëdes lineatopennis* could be

obtained from the same source but were not present in such large numbers. Besides these two species, *A. dentatus* and *A. vittatus*, hatched from captured larvae, were available, the former species in sufficiently large numbers but the latter being represented by only a few specimens.

We were able to feed the following mosquitoes on the virus horse:—

Aëdes caballus	816 specimens.
Aëdes lineatopennis	151 specimens.
Aëdes dentatus	61 specimens.
Aëdes vittatus...	7 specimens.

From the epidemiological point of view the former two species had to be regarded as the more probable natural transmitters.

Experimental Technique.—During the course of these experiments the finally adopted technique was already being employed as far as possible. The mosquitoes were fed on the virus horse in the mosquito tent, which was kept wet by two showers running all night throughout the duration of the experiments. The special stable was still under construction at that time, but the saddle for keeping the small mosquito cages in position, described elsewhere, had already been completed.

When the time arrived for the mosquitoes to be fed on the susceptible horses the special stable had been completed and we used it in preference to the mosquito tent.

A. caballus was, as a rule, difficult to keep alive in the laboratory for long periods; especially was this the case with the fertilized specimens caught as adults, large numbers of which died after oviposition. We were, furthermore, exceptionally unlucky at this time on account of our losing hundreds of specimens as a result of an unforeseen invasion of ants into the cages. Of more than 800 specimens only 100 were left after two weeks, and at the end of the following week this number had been reduced to not more than about 10.

In the case of *Aëdes lineatopennis* the results were much better. Of 151 specimens which had fed on the virus horse, 71 or nearly 50 per cent. were alive after 19-21 days, 38 specimens after slightly more than a month (33-35 days), and 11 specimens were still present after as long an interval as 60-62 days. This may certainly be regarded as satisfactory.

The original material of *A. dentatus* consisted of 61 specimens, of which 22 specimens survived after 25-27 days, and 6 after 35-37 days.

Only 7 specimens of *A. vittatus* had fed on the virus horse and these proved to be very resistant to the experimental conditions.

Only in the case of *A. caballus* were the feeding results not very satisfactory and this could be attributed to the horse, which was in rather poor condition, having an extremely hollow back, which resulted in the under surface of the cages not coming into proper contact with skin and, therefore, not allowing of the mosquitoes getting at the animal properly.

In the experiments with *A. lineatopennis*, for example, 51 out of 71 fed after an interval of 19-21 days, 33 out of 42 after 25-27 days, 33 out of 38 after 33-35 days, and 10 out of 11 specimens after 60-62 days. In the case of *A. dentatus* 20 out of 22 specimens fed after 25-27 days.

C. Experiments with *Aëdes caballus*.

We succeeded in feeding more than 800 specimens of *A. caballus* on the virus horse but, owing to a large mortality already referred to above, only 100 specimens remained at the end of a fortnight, of which about 70 could be induced to feed again on our experimental horse. The results must, therefore, be regarded as fairly satisfactory.

Virus horse 10 was used, and mosquito groups:—

Group 20.—Fed on virus horse 10 during the night of 9th to 10th March, 1932. First day of fever. Temperature 103·7°-102·5°. 181 specimens engorged (reared from larvae). Used for experiment 19.

Group 21.—Fed together with group 20. 26 specimens engorged (caught as adults). Used for experiment 19.

Group 22.—Fed on the same virus horse during the following night (10th-11th March). Second day of fever. Temperature 103·0°-104·6°. 156 specimens engorged (reared from larvae). Used for experiment 19.

Group 23.—Fed together with group 22. 242 specimens engorged (caught as adults). Used for experiment 19.

Group 25.—Fed on the same virus horse one night later (11th to 12th March). Third day of fever. Temperature 106·3°-105·3°. 211 specimens engorged (caught as adults). Used for experiment 19.

Experiment 19 (*H.S.* 24). *Aëdes caballus. Feeding. Interval* 14-20 *days. Horse* 20297.

This horse had been used about 4 months previously in experiment 5 (O-virus, *Aëdes vittatus*, injection, interval 15 days), but had shown no temperature reaction following the injection of mosquitoes.

On 23rd March, 1932, the combined groups 20 and 21 of *A. caballus* were put on to the horse, and during the night 33 (out of 44) specimens engorged themselves. The next day the unfed mosquitoes of these groups were allowed to feed again during the daytime and five more specimens took up blood. During the following night (24th to 25th March) groups 22 and 23 were allowed to feed, and 27 (out of 42) specimens engorged themselves. On 26th March six (out of 19) specimens of group 25 fed. On the night of 29th to 30th March the remainder of group 20 and 21 were put on to the horse again and six (out of 10) specimens fed.

In all, the horse was bitten 77 times by mosquitoes as follows:—

33 specimens (group 20 and 21) after an interval of 14 days.
5 specimens (group 20 and 21) after an interval of 15 days.
27 specimens (group 22 and 23) after an interval of 14 days.
6 specimens (group 25) after an interval of 14 days.
6 specimens (group 20 and 21) after an interval of 20 days.

Or, in other words, 71 specimens after 14-15 days and by six after 20 days. These mosquitoes had fed on the virus horse during the first to third day of the fever reaction.

Reaction: The horse was kept under observation until 10th April, i.e. 42 days after the last group of mosquitoes had fed. It showed no fever reaction whatsoever the highest temperature recorded during this period being 101°.

Immunity Test: On 11th April the horse was injected intrajugularly with 5 c.c. blood of the original Losperfontein virus. The temperature remained normal for 5 days, up to 16th April, when it was followed on the ensuing day by a slight rise up to 101°. Only the day after did the definite fever reaction commence, temperatures of 100·6° and 103·8° being recorded on 18th April and 103·2° and 104·2° on the 19th. The following night, 8-9 days p.i., the horse died.

Result: This experiment must be regarded as negative. No temperature reaction followed the feeding by the mosquitoes, but after the intrajugular injection of the same strain of virus the horse succumbed to horsesickness within 9 days. The incubation period of 6 days in this case was relatively long, especially when compared with that following an injection of the O-virus strain, but this prolongation cannot be regarded as an indication of a slight immunity, as we experienced a similar long incubation period (5 days) in the case of the virus horse injected with the same material.

D. Experiments with *Aëdes lineatopennis*.

Of *A. lineatopennis* a smaller number than was the case with *A. caballus* was available for feeding on the virus horse, viz., only about 150 specimens. On the other hand, we were more successful in keeping them alive as, after 20 days 71 specimens survived and after more than two months 11 specimens were still living.

Virus horse 10 was used, and the following mosquito groups:—

Group 17.—Fed on virus horse 10 during the night of 9th-10th March, 1932. First day of fever. Temperature 103·7° and 102·5°. 26 specimens engorged (reared from larvae). Used for experiments 20 and 21.

Group 18.—Fed together with group 17. 69 specimens (caught as adults) engorged. Used for experiments 20 and 21.

Group 19.—Fed on the same virus horse during the following night (10th-11th). Second day of fever. Temperature 103·0°-104·6°. 22 specimens engorged (reared from larvae). Used for experiments 20 and 21.

Group 20.—Fed on the same virus horse one night later (11th-12th March). Third day of fever. Temperature 106·3° and 105·3°. 10 specimens engorged (caught as adults). Used for experiments 20 and 21.

Group 21.—Fed together with group 20. 11 Specimens engorged (reared from larvae). Used for experiments 20 and 21.

Experiment 20 (*H.S.* 22). *Aëdes lineatopennis. Feeding. Interval* 13-62 *days. Horse* 20276.

On 22nd March, 1932, the mosquitoes of group 18 were put on to this horse for feeding and 35 (out of 55) specimens engorged themselves during the night. The unfed specimens of this group were allowed to feed again on 26th March, during the day and 3 (out of 8) specimens engorged themselves. On 30th March the remaining mosquitoes of the combined groups 17 to 21 were put on to the horse and during the night, of the 71 specimens, 50 fed. The same mosquitoes were fed again during the night of 6th to 7th April and 33 (out of 42) took up blood. Eight days later, on 13th April, the 38 mosquitoes still alive were fed and once more 33 specimens engorged themselves during the ensuing night. Almost one month later we still had 11 specimens, and of these, 10 fed for the last time during the night of 10th to 11th May.

In all, this horse was bitten 165 times by the mosquitoes as follows:—

35 specimens (group 18) after an interval of 13 days;
3 specimens (group 18) after an interval of 17 days;
51 specimens (group 17-21) after an interval of 19-21 days;
33 specimens (group 17-21) after an interval of 25-27 days;
33 specimens (group 17-21) after an interval of 33-35 days;
10 specimens (group 17-21) after an interval of 60-62 days;

or in other words by 38 specimens after 13-17 days, 51 after 19-21 days, 66 after 25-35 days, and by 10 after 60-62 days. These mosquitoes had all fed on the virus horse during the first to the third day of fever.

Reaction: After the first feed of the mosquitoes on 22nd March the temperature, to start with, remained normal for 22 days, up to 13th April, the maximum being 101°. On 14th April the temperature rose suddenly from 99° in the morning to 103·2° in the afternoon. The following day, however, it returned to normal, where it remained for the succeeding 2 days. During the next 4 days irregular temperatures were recorded, viz., on the 18th, 98 and 101°, the 19th, 98·4 and 100·5°, the 20th, 98·6 and 102°, and on the 21st, 98·4 and 101·6°. Thereafter the temperature remained normal (below 100·5°) for 42 days, up to 3rd June, 23 days after the feeding of the last group of mosquitoes.

Immunity test: On 3rd June the horse received 1 c.c. blood of the original Losperfontein case subcutaneously. Up to 17th June, 14 days after the injection, no fever reaction occurred, the highest temperature recorded being 100·8°.

On 17th June the horse received a further injection of the same material, but this time 2 c.c. intrajugularly. The temperature remained normal for 6 days following the second injection (maximum 100°). On the 7th day, 24th June, temperatures of 99 and 101° were recorded, the 25th 101 and 102°, the 26th (a.m.) 103·6°, and the 27th 104·4 and 104·2°. The horse was then transferred to another experiment wherein it received an intravenous injection of mercurochrome. It died the following day with typical symptoms of dunkop.

Result: There seems little doubt but that this experiment must be regarded as *negative*. Notwithstanding the fact that this horse was bitten 165 times by mosquitoes of different groups, no definite fever reaction occurred. Except for the one brief rise to 103·2° the temperature was slightly higher than normal only between 18th and 21st April (maximum 102°) and this was not sufficient to justify its being regarded as a weak reaction. The result of the immunity test was undoubtedly unusual. The first injection of 1 c.c. of infected blood, performed subcutaneously, produced no result, at any rate during the observation period of 14 days, which was perhaps somewhat short. The following injection of 2 c.c. of blood intrajugularly was followed by the death of the horse, the incubation period of the disease of 7-8 days being relatively long. It is not impossible that the horse possessed a slight natural immunity or an immunity acquired following the mosquito bites and that this immunity was sufficient to protect it against a subcutaneous injection of 1 c.c. of virus, but was subsequently broken down by the intravenous injection of 2 c.c. of virus, only the lengthened incubation period being indicative of this initial immunity. However, the observation period following the subcutaneous injection was unfortunately somewhat short and, moreover, there may be other explanations, e.g. failure of the injection with stored blood to set up the disease which is occasionally met with, to account for the apparent failure of this initial injection, so that we, therefore, prefer, as already stated, to regard this experiment as *negative.*

Experiment 21 (*H.S.* 36). 6 *Aëdes lineatopennis. Injection. Interval* 63-65 *days. Horse* 20236.

In the preceeding experiment it was noted that of the combined groups 17-21 of *Aëdes lineatopennis* 11 specimens were still alive more than two months after their original feed on an infected virus horse. Of these mosquitoes 10 specimens fed for the last time during the night of 10th to 11th May.

On 13th May, 1932, there were still 6 specimens of these groups alive. These were emulsified in normal horse serum and injected into horse 20236. On the date of injection 63 to 65 days had elapsed since these mosquitoes had had their initial feed on the infected virus horse.

Reaction: The morning following the injection the temperature rose to 102° but regained normal the next day. The horse was kept under observation for 31 days, up to 13th June, but no typical fever reaction resulted throughout this period. On two occasions only did the temperature exceed 101° but these fluctuations were not maintained for more than 24 hours in each case.

Result: The experiment must be regarded as *negative* as the injection of the mosquitoes was not followed by a temperature reaction. The immunity test of this horse by virus injection was not considered to be necessary.

E. Experiments with *Aëdes dentatus*.

In addition to the *Aëdes caballus* and *A. lineatopennis*, mentioned above, 61 specimens of *A. dentatus* were fed on the virus horse infected with the Losperfontein strain virus and used in the previous experiments. The mortality amongst these mosquitoes was not too high, one third of the original number surviving after 25-27 days. For this experiment the same virus horse was used, viz.:—

Virus horse 10 and mosquito groups:—

Group 11.—Fed on virus horse 10 during the night of 9th to 10th March, 1932. First day of fever. Temperature 103·7-102·5°. 21 specimens engorged (reared from larvae). Used for experiment 22.

Group 12.—Fed on the same virus horse during the following night (10th-11th March). Second day of fever. Temperature 103·0-104·6°. 28 specimens engorged (reared from larvae). Used for experiment 22.

Group 13.—Fed on the same virus horse one night later (11th-12th March). Third day of fever. Temperature 106·3-105·3°. 12 specimens engorged (reared from larvae). Used for experiment 22.

Experiment 22 (*H.S.* 28). *Aedes dentatus. Feeding. Interval* 25-37 *days. Horse* 20314.

On 5th April, 1932, the combined groups 11 to 13 of *Aedes dentatus* were put on to the horse, and during the following night 20 (out of 22) specimens engorged themselves. The remaining mosquitoes of the same groups were fed again on the same horse 10 days later, during the night of 15th-16th April, and 6 specimens took up blood.

The horse was therefore bitten 26 times by the mosquitoes as follows:—

20 specimens (group 11-13) after an interval of 25-27 days.

6 specimens (group 11-13) after an interval of 35-37 days.

These mosquitoes had had their initial feed on a virus horse during the first to third day of fever.

Reaction: The horse was kept under observation up to 16th May, i.e. one month after the last feeding of the mosquitoes. The temperature exceeded 101° on each of the two days on which the mosquitoes fed, viz., on 9th and 19th, April, but was not maintained for longer than half a day in each case, and the highest temperature registered was only 101·4°. Apart from these small fluctuations the temperature remained between 98 and 101°.

Result: The experiment has to be regarded as *negative*, as no temperature reaction of any consequence followed the feeding of the mosquitoes during an observation period of one month. In this case as well, the horse was not tested for immunity.

F. Experiments with *Aëdes vittatus*.

A. vittatus was only obtainable by us in very small numbers at the time of these experiments. Not more than 7 specimens fed on the virus horse but the mortality amongst them was low and 4 mosquitoes were still alive at the end of one month.

In this experiment we used virus horse 10 and mosquito group:—

Group 3.—Fed on virus horse 10 during the night of 9th-10th March, 1932. First day of fever. Temperature 103·7-102·5°. 7 specimens engorged (reared from larvae). Used for experiment 23.

Experiment 23 (*H.S.* 26). *Aëdes vittatus. Feeding. Interval* 23-37 *days. Horse* 20298.

On 1st April, 1932, *Aëdes vittatus* group 3 was put on to the horse of this experiment, 5 specimens taking a blood feed during the night. The same mosquitoes were fed again on this horse during the night of 15th-16th April, when 4 specimens engorged themselves.

The horse was bitten therefore 9 times, viz., by 5 specimens after an interval of 23 days, 4 specimens after an interval of 37 days. The mosquitoes had fed on the virus horse during the first day of its fever reaction.

Reaction: The horse was kept under observation until 16th May, i.e. one month after the feeding of the last mosquitoes. During this period only once, viz., on the day following the second feed of the mosquitoes, was there a rise in temperature exceeding 101°, when 101·5° was recorded, but this was not maintained for longer than half a day. With the exception of this insignificant elevation the temperature varied between 98·4 and 100·9°.

Result: As no fever reaction whatsoever followed the feeding of the mosquitoes, the experiment must be regarded as *negative*. The immunity test was omitted in this case, as in the preceding experiment.

G. Discussion of the Results Obtained with Losperfontein Virus.

On a horse infected by means of a blood inoculation from a mule at Losperfontein showing typical symptoms of dikkop, 816 *Aëdes caballus*, 151 *A. lineatopennis*, 61 *A. dentatus* and 7 *A. vittatus* were fed. These mosquitoes were kept in a room during the experiment in which the temperature varied between 20 and 30° C. with a probable mean of 24° C. They were fed on susceptible horses commencing at 13-14 days after their initial feed and this feeding was continued at intervals, in some cases up to more than two months. Injection of emulsified mosquitoes was only exceptionally employed in this series of experiments.

The results of these experiments were as follows:—

Aëdes caballus (experiment 19). Interval 14-15 days: 71 specimens. Interval 20 days: 6 specimens. Result negative.

Aëdes lineatopennis (experiment 20):

Interval 13-17 days: 38 specimens.
Interval 19-21 days: 51 specimens.
Interval 25-27 days: 33 specimens.
Interval 33-35 days: 33 specimens.
Interval 60-62 days: 10 specimens.
Total, 165 specimens.

Result: Negative.

Aëdes lineatopennis (experiment 21) injection.

Interval 63-65 days: 6 specimens.

Result: Negative.

Aëdes dentatus (experiment 22).

Interval 25-27 days: 20 specimens.
Interval 35-37 days: 6 specimens.

Result: Negative.

Aëdes vittatus (experiment 23).

Interval 23 days: 5 specimens.
Interval 37 days: 4 specimens.

Result: Negative.

The four horses together were bitten 277 times by the four *Aëdes* species after the following intervals:—

Interval 13-17 days: 109 specimens.
Interval 19-21 days: 57 specimens.
Interval 23-27 days: 58 specimens.
Interval 33-37 days: 43 specimens.
Interval 60-62 days: 10 specimens.

In none of these experiments were positive results or fever reactions, which might be suspected of being possible reactions, obtained. In the first two experiments the horses were tested for immunity by injection of the same strain. In the first case the injection was performed intrajugularly and followed in almost the normal time by the death of the horse with typical symptoms. In the second case a subcutaneous injection of 1 c.c. of blood containing virus was apparently a failure, but a second, and in this case intrajugular injection, produced a fatal infection after a somewhat prolonged incubation period. However, we regard this horse also as being normally susceptible, the reasons for which were given under the discussion of the results of the experiment in question. The other three horses were not tested for immunity, this not being regarded as absolutely necessary in view of the fact that the other horses from the same lot tested in various experiments proved to be susceptible.

What was the cause of the failure of these experiments? There is first of all naturally the possibility that none of the mosquito species used was the actual transmitter. We have already, on several occasions stated why these species were chosen for our experiments.

The numbers of mosquitoes used may have been insufficient. This, however, does not appear to be the case when the data of our experiments is taken into consideration, and it is furthermore improbable that the natural transmitter would have a very low infection index.

The time allowed for the extrinsic incubation period may have been insufficient, but, in our experiments, in the case of quite a number of mosquitoes used for feeding at any rate, it was certainly more than sufficient as the horses were bitten more than 100 times by mosquitoes which had fed more than three weeks previously, and 10 times where the interval exceeded two months.

The temperature at which the mosquitoes were kept is also considered to have been high enough for a normal development of the virus in the mosquitoes. Neither in other questions of minor importance could a reason for the failure of these experiments be found. One important possibility, however, still remains, as already pointed out in the introductory chapter, viz., that the Losperfontein virus, derived from a mule previously immunized with the O-virus strain, was nothing else than O-virus. We will leave the question of the possibility of a relapse in horsesickness out of the discussion at this stage and only lay stress on the fact that there existed a very striking resemblance between the fever reactions set up by our Losperfontein strain and the O-virus used in the immunization of the mules. The strain was obtained in a place where quite a large number of animals had been injected previously with O-virus and should the two strains be identical the objections against the use of O-virus for transmission experiments, discussed fully in one of the previous chapters, would apply equally well in the case of Losperfontein virus.

VIII. EXPERIMENTS WITH ESHOWE-VIRUS.

Towards the end of February, 1932, almost at the same time that the Losperfontein strain was obtained, Mr. Franz, the Government Veterinary Officer of Eshowe, Zululand, sent us blood from a mule, which had died from horsesickness. We are very much indebted to Mr. Franz for the trouble he took in forwarding us a fresh strain from his area.

The mule from which the strain was derived, referred to here as the Eshowe strain, suffered from a typical attack of " dikkop ", followed by recovery. It had been immunized in August, 1931, that is about 6 months previously, with the O-virus strain always used for this purpose.

The first horse (20302) injected with this strain showed a course of infection somewhat different from that of the ordinary O-virus type; a longer incubation period and a fever more of the remittent type. By the injection of mosquitoes fed on this animal into a susceptible horse (20288) an infection was produced which, except for a longer incubation period, to be expected, resembled more closely the O-virus course. Besides these two horses 9 more were injected with the Eshowe-virus, 3 as virus horses, 5 for testing the immunity, and 1 for a reason outside our work.

In the case of the three virus horses, viz., 20270, 20299 and 20310, the first showed an acute infection commencing two days after injection with death on the fifth day, of mixed horsesickness. In the second case a reaction set in on the second day after injection with death on the afternoon of the seventh day of mixed horsesickness. The third animal commenced reacting on the afternoon of the fifth day after injection with death on the tenth day, again in this case of mixed horsesickness. The 5 horses of which the immunity was tested by means of injections of Eshowe-virus all reacted positively and died of acute horsesickness. These horses were: 20300, 20315, 20291, 20280, 20130. The incubation periods varied between 4 and 5 days, and the duration of the reactions between 3 and 4 days, three of the horses dying from the " dikkop " and 2 from the " dunkop " form of the disease. The clinical picture of the reactions in the above 8 horses is undoubtedly somewhat different from what is experienced in the case of O-virus where after an incubation period of 2 to 3 days and a duration of an additional 2 days horses almost invariably succumb to the " dunkop " form of the disease. However, in comparing these reactions with those set up by most " field " viruses distinct differences are noticeable. Whereas the incubation period of the " field " virus varies as a rule between 6 to 8 days and the peak period of the reaction is reached in 3 to 4 days by a series of remissions, the above reactions apart from a slightly increased incubation period incline more closely to the O-virus type of reaction with a rapid rise to the height of the reaction followed by virtual collapse. This strain was not what we really wanted as it was derived from an animal previously immunized, as was also the case with the Losperfontein strain. We therefore still had to wait for a genuine natural strain which, however, was not obtained until May, when there was scarcely any opportunity left of conducting any more

experiments. In the meantime we completed as many experiments as possible with the Eshowe-strain which we regarded as the best strain we had at our disposal, as it showed at any rate some difference from the O-virus.

In all, 9 experiments were made, in which *Aëdes caballus*, *A. lineatopennis*, *A. hirsutus* and *A. dentatus* were used. 280 specimens were injected after an interval of 7-30 days and 379 mosquitoes induced to feed after an interval of 15-22 days on the susceptible horses.

A. Virus Horses.

In the experiments with the Eshowe strain 4 horses were used for feeding mosquitoes on; one injected with the original material, 2 with the first and one with the second laboratory generation.

Virus horse 11 (*horse* 20302).—Injected on 25th February, 1932, intrajugularly with 5 c.c. blood of a natural case of horsesickness in an immunized mule at Eshowe.

Result: Temperature normal up to 29th February p.m. Temperature on 1st March, 101·2 and 103·0°: the 2nd, 102·0 and 104·8°: the 3rd, 103·2 and 105·5°; the 4th, 103·9 and 103·3°; and the 5th (a.m.), 99·2°. The horse died during the same day. Post-mortem diagnosis: Horsesickness, dunkop. It was an acute infection with a relatively long incubation period of 5 days.

Virus horse 12 (*horse* 20288).—Infected in experiment 24 by injection of mosquitoes (for temperature see under experiment 24).

Virus horse 13 (*horse* 20270).—Injected on 19th March, 1932, intrajugularly with 5 c.c. blood of virus horse 11.

Horse 13 had been used one month before in experiment 17 (O-virus, *Aedes* spp., injection, interval 7-8 days), with negative results.

Result: Temperature normal up to 21st March p.m. Temperature on 22nd March, 100·6 and 103·1°: the 23rd, 99·5 and 104·5°; and the 24th, 103·0° (a.m.). The horse died during the following night. Post-mortem diagnosis: Horsesickness, mixed. The course of the disease was very acute, resulting in death within 5 days p.i., with the temperature relatively low. The incubation period was only 2 days in this case.

Virus horse 14 (*horse* 20299).—Injected on 16th April intrajugularly with 5 c.c. blood of virus horse 11.

Result: Temperature normal for two days p.i.: on 19th April, 100 and 103·2°; the 20th, 101·0 and 102·0°; the 21st, 102·0 and 101·4°; the 22nd, 101·7 and 103·1°; the 23rd, 103·0 and 104·6°. The horse died during the following night. Post-mortem diagnosis: Horsesickness, mixed. The case was acute with an incubation period of 3 days.

Virus horse 15 (*horse* 20310).—Injected on 18th April with 10 c.c. serum of virus horse 12, which had been cataphorized by the passage of an electrical current of 9 milliamperes at 250 volts for 4 hours.

Result: Temperature rose to 104° on the day of injection, but returned to normal the next day. On 23rd April, 101·2° was registered. This was maintained on the 24th. The 25th, 102·6 and 104° were recorded: the 26th, 104·6 and 105°; the 27th, 104 and 106°. On the 28th the temperature dropped to 103° and the horse died during the day, the post-mortem findings revealing the mixed form of horsesickness. Acute horsesickness with an incubation period of 6 days had resulted. It is doubtful whether the passage of the electrical current had any effect upon the potency of the virus and the somewhat lengthened incubation period is therefore somewhat difficult to account for.

B. Mosquitoes and Experimental Technique.

Mosquitoes.—From the beginning of March, when these experiments were commenced, till the end of April, notwithstanding the dry season, *Aëdes caballus* could be obtained in quite large numbers, either as larvae or adults, by artificial flooding of their breeding

places. *Aëdes lineatopennis*, the species we were most anxious to test thoroughly, was still present in sufficient numbers at the beginning of March. They then, however, became more rare, being outnumbered in their breeding places by *A. caballus*. By the end of March our supply was practically exhausted. During the first experiment with this strain *Aëdes hirsutus* was fortunately hatching out in large numbers from one of the breeding places, but thereafter this species also became too scarce for experimental purposes. *Aëdes dentatus* was never really common during the period we were working with this strain, but a fairly regular supply could at any rate always be obtained.

On the virus horse the following mosquitoes were fed:—

Aëdes caballus	1,068 specimens.
Aëdes lineatopennis	272 specimens.
Aëdes hirsutus	211 specimens.
Aëdes dentatus	129 specimens.

In our opinion, as arrived at during the mosquito survey, the probable transmitters of horsesickness had to be looked for amongst the three first-named species.

Experimental Technique.—The technique described in the second paper as that finally adopted, was already in use as far as possible from the commencement of these experiments.

The fresh mosquitoes were fed on virus horses 11, 13 and 15 in the mosquito tent, the provisional arrangement for feeding mosquitoes, whereas in the case of virus horses 12 and 14 the specially constructed stable was used.

For feeding the mosquitoes on susceptible horses our special stable was used for preference and the tent only resorted to when the stable was already in use for another experiment.

After their blood-meal on one of the virus horses the mosquitoes were kept in our warm room, *A. caballus*, *A. lineatopennis*, and *A. hirsutus* in the larger cages covered with wet hessian, and *A. dentatus* mainly in small jars.

Aëdes caballus was again the most sensitive to experimental conditions, showing the highest mortality of all the species. Out of 132 specimens used for injection about 50 per cent. were alive one week after the first feeding, and out of more than 900 specimens, which were used in feeding experiments, only about 10 per cent. survived 15-21 days. The highest mortality occurred as usual at the end of the first week after the initial feeding when eggs were deposited, although on this occasion most of the groups consisted of specimens reared from larvae, eggs were frequently laid. Females and males had hatched out together in the large cages, and before being used had remained there together for some time during which copulation must have taken place.

The results with *A. lineatopennis* after longer intervals were as usual better than with the former species. Out of 163 specimens used for injection 66 (or 10 per cent.) were alive after one week, showing, therefore, a mortality slightly higher than in the case of *A. caballus*. However, out of 109 engorged females from the feeding

experiment 49 specimens, or nearly 50 per cent., were alive 16-18 days after their initial feeding. This species does not seem to be affected to the same extent by artificial conditions as *A. caballus*.

Aëdes hirsutus once more proved to be very resistant to the experimental conditions under which they were kept. In fact, it was the easiest species to handle. Out of a total of 311 specimens 100 were injected after 7 days. Of the remaining 211 specimens 107 were alive at the end of 20-21 days, and 46 after 29-30 days, thus giving results that may be looked upon as very satisfactory. In this case copulation had not taken place.

With *Aëdes dentatus* the results were quite good, although not as good as with *A. hirsutus*. Originally 129 specimens had fed and of these nearly 50 per cent. were still alive at the end of 14-21 days.

C. Experiment with *A. caballus* and *A. lineatopennis*.

In the first experiment with the Eshowe virus a number of *A. caballus* and *A. lineatopennis* were injected together into the same horse after an interval of 7 days. Another horse was injected under the same conditions with *A. hirsutus*.

At the time of these experiments no larvae of *A. caballus* or *A. lineatopennis* were at our disposal, and we had to make use of specimens freshly caught in the field where they could be obtained in sufficient numbers.

Virus horse 11, injected with the original Eshowe material, was used for feeding mosquitoes on, and mosquito groups:—

A. caballus group 17.—Fed on virus horse 11 during the night of 1st to 2nd March. First day of fever. Temperature 103·0-102·0°. 21 specimens (caught as adults) engorged. Used for experiment 24.

A. caballus group 18.—Fed on the same virus horse during the following night. Second day of fever. Temperature 104·0-103·2°. 34 specimens engorged (caught as adults). Used for experiment 24.

A. caballus group 19.—Fed on the same virus horse one night later (3rd to 4th March). Third day of fever. Temperature 105·5-103·9°. 77 specimens engorged (caught as adults). Used for experiment 24.

A. lineatopennis group 12a.—Fed together with *A. caballus* group 17. 35 specimens engorged (caught as adults). Used for experiment 24.

A. lineatopennis group 13.—Fed together with *A. caballus* group 18. 57 specimens engorged (caught as adults). Used for experiment 24.

A. lineatopennis group 14.—Fed together with *A. caballus* group 19. 71 specimens engorged (caught as adults). Used for experiment 24.

Experiment 24 (*H.S.* 20). 68 *A. caballus and* 66 *A. lineatopennis. Injection. Interval* 7 *days. Horse* 20288.

This horse was injected with 68 *A. caballus* and 66 *A. lineatopennis*, altogether 134 specimens. On 9th March, 1932, 14 *A. caballus* group 17 were injected and 16 *A. lineatopennis* group 12a; on 10th March, 16 *A. caballus* group 18 and 22 *A. lineatopennis* group 13; on 11th March, 38 *A. caballus* group 19 and 28 *A. lineatopennis* group 14. All these mosquitoes had fed on an infected horse 7 days before, during the first to third days of its fever reaction.

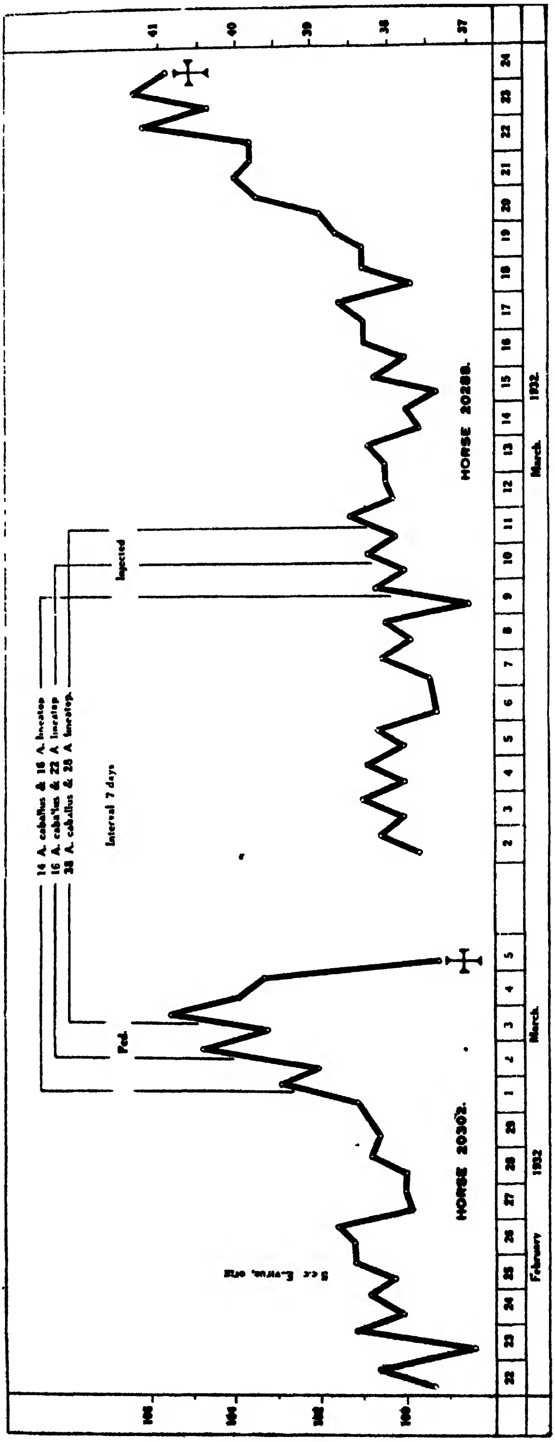
HORSE 20288.
HORSE 20302.
Injected
Fed.
Interval 7 days
March
February
1932

Fig. 3.

Reaction (*vide* fig. 3): The temperature remained practically normal up up 18th March, 9 days after the injection of the first group of mosquitoes and 7 days after the last. On the following days the temperature was: 19th March, 101·0 and 101·7°; the 20th, 102·0 and 103·5°; the 21st, 104·0 and 103·6°; the 22nd, 103·6 and 106·2°; the 23rd, 104·5 and 106·5°; and the 24th (a.m.), 105·5°. During the afternoon of the same day the horse died. The post-mortem diagnosis was: horsesickness, dunkop.

The *result* of this experiment was *positive*. Virulent virus was, therefore, present in one or more of 134 *A. caballus* and *A. lineatopennis* 7 days after their having fed on an infected horse.

D. Experiments with *Aëdes caballus*.

The experiments with this species were carried out from the latter part of March until the middle of May, 1932. *Aëdes caballus* could be obtained relatively easily, much more easily in any case than the other species. Altogether more than 900 specimens were fed on the virus horses. Owing to a considerable mortality following upon oviposition, however, only 83 specimens fed for a second time on the susceptible horses.

Four experiments were made with this species; experiment 25, with mosquitoes fed on virus horse 12, which had been infected by the injection of mosquitoes after an interval of one week; experiment 26, with specimens from virus horse 14; experiment 27, with specimens from virus horse 15, and experiment 28, with mosquitoes fed on horse 20300 from experiment 25, during a fever reaction which might have been of the nature of horsesickness fever.

The mosquitoes were:—

Group 25*a*.—Fed on virus horse 12 during the night of 21st to 22nd March, 1932. Second day of fever. Temperature 103·6-103·6°. 260 specimens engorged (caught as adults). Used for experiment 25.

Group 27.—Fed on the same horse the following night. Third day of fever. Temperature 106·2-104·5°. 192 specimens engorged (caught as adults). Used for experiment 25

Group 31.—Fed during the night of 18th-19th April on horse 20300 of experiment 25. Temperature 102·4-101·2°. 86 specimens engorged (reared from larvae). Used for experiment 28.

Group 32.—Fed on virus horse 14 during the night of 19th-20th April. First day of fever. Temperature 103·2 and 101°. 104 specimens engorged (reared from larvae). Used for experiment 26.

Group 33.—Fed during the night of 19th-20th April on horse 20300 of experiment 25. Temperature 103·6-101·4°. 130 specimens engorged (reared from larvae). Used for experiment 28.

Group 35.—Fed on virus horse 14 during the night of 21st and 22nd April. Third day of fever. Temperature 101·4-101·7°. 107 specimens engorged (reared from larvae). Used for experiment 26.

Group 36.—Fed on virus horse 15 during the night of 26th-27th April. Fourth day of fever. Temperature 104-106°. 57 specimens engorged (reared from larvae). Used for experiment 27.

Experiment 25 (*H.S.* 30). 10 *Aëdes caballus*. *Feeding*. *Interval* 16-17 *days*. *Horse* 20300.

On 7th April, 1932, *A. caballus* groups 25a and 27 were put on to this horse and 10 specimens fed during the night. These mosquitoes had had their initial feed 16-17 days previously, during the second and third days of fever, on a horse infected by the injection of mosquitoes.

Reaction: This horse normally showed a fairly high temperature, which varied during the week preceding the application of the mosquitoes between 100 and 102°, averaging 101° for the period.

The day after the feeding of the mosquitoes an attack of colic occurred and the temperature rose to 104·6°, but dropped back to normal (100°) the following day. It remained below 102° for three more days when a febrile reaction commenced. The temperature on 13th April was 101·2 and 102·4°; the 14th, 100 and 102·0°; the 15th, 101·1 and 101·2°; the 16th, 102·4 and 104·3°; the 17th (a.m.) 102°; the 18th, 103 and 102·4°; the 19th, 101·2 and 103·6°; the 20th 101·4 and 102·2°. From 21st April to 10th May the temperature only once reached 102° for half a day, viz., on 7th May, for the rest it remained between 100 and 101·5°.

Subinoculation: On 19th April, 1932, 5 c.c. blood of horse 20300, taken the day before, during the slight febrile reaction, was injected intrajugularly into horse 20309. During an observation period of one month no febrile reaction occurred, the highest temperature registered being 100·4°, excluding the day of the injection and the day following thereupon. It was not thought necessary to test the susceptibility of this horse by virus injection.

Testing by means of mosquito feeding: During the febrile reaction two groups of *A. caballus* were fed on the horse and about 20 days after were again fed on a susceptible horse, but with negative results. A full account will be given under experiment 28.

Immunity test: On 11th May, 33 days after the feeding of the mosquitoes and 21 days after the end of the febrile reaction, the horse was injected intrajugularly with 5 c.c. blood of horse 20288 (virus horse 12). Five days later, on 16th May, the temperature rose to 105·4° and remained between 105 and 105·8° during the following 2 days. On the next morning, 19th May, the temperature was 102·2° and the horse died the same day.

Result: This experiment must also be regarded as negative. Eight days after the feeding of the group of mosquitoes a fever reaction occurred which might have been due to a slight attack of horsesickness fever. A subinoculation of blood into a normal horse was negative, however, and 3 weeks after this reaction the horse proved to be normally susceptible when injected intrajugularly with the same strain.

Experiment 26 (*H.S.* 34). *Aëdes caballus. Feeding. Interval* 18-20 *days. Horse* 20331.

On 9th May, 1932, *A. caballus* groups 32 and 35 were put on to horse 20331 and during the ensuing night 14 specimens of group 32 and 4 of group 35 engorged themselves. In total, therefore, the horse was bitten by 18 specimens, viz., by—

4 specimens (group 35) after an interval of 18 days;
14 specimens (group 32) after an interval of 20 days.

These mosquitoes had fed on the infected horse during the first and third days of fever.

Reaction: The horse was kept under observation up to 12th June, 35 days after the feeding of the mosquitoes. Only once during this period did the temperature reach 101·0°, and then only for half a day.

Result: As no temperature reaction whatsoever followed the biting of the mosquitoes the experiment has to be regarded as *negative*. The test for immunity was omitted in this case.

Experiment 27 (*H.S.* 35). *Aëdes caballus. Feeding. Interval* 15 *days. Horse* 20333.

On 11th May, 1932, *A. caballus* group 36 was put on to this horse, but only three specimens took up blood. These mosquitoes had fed 15 days previously on virus horse 15, which had been infected with a strain of virus obtained from virus horse 12. This virus prior to injection, had been subjected to cataphorisation by the passage of an electrical current of 9 milliamperes under a pressure of 250 volts for 4 hours.

Reaction: The temperature of this horse remained normal for 33 days, after the feeding of the mosquitoes (up to 13th June), when the horse was discharged from observation Only once did the temperature exceed 101° for half a day.

The result of this experiment was negative, as no reaction at all occurred after the feeding of the mosquitoes. A test of the susceptibility of the horse was not thought necessary.

Experiment 28 (*H.S.* 33). *Aëdes caballus. Feeding. Interval* 20-21 *days. Horse* 20235.

On 9th May, 1932, *Aëdes caballus* groups 31 and 33 were put on to this horse and during the following night 52 (out of 66) specimens engorged themselves. These mosquitoes had fed 20-21 days previously on horse 20300 of experiment 25 during a fever reaction which, as far as one could see at the time, might have been connected with horsesickness fever.

Reaction: The temperature at first remained normal for 14 days. A slight fever reaction then commenced. The temperature on 24th May was 101·4 and 101·6°; the 25th, 102 and 101°; the 26th, 103·4 and 103·6°; the 27th, 102·8 and 102·4°. From the 28th onwards the temperature was normal again up to 13th June, when the horse was discharged from observation.

Result: We will regard this experiment also as *negative*. About two weeks after the feeding of the mosquitoes a slight temperature reaction occurred, but we cannot regard this as an attack of horsesickness fever as the horse on which these mosquitoes had had their initial feed was not infected with horsesickness as shown by the immunity test in experiment 25. For the same reasons a test of the susceptibility of this horse was not thought necessary.

E. Experiment with *Aëdes lineatopennis*.

Together with the first groups of *Aëdes caballus* referred to in the preceding section, 89 *Aëdes lineatopennis* were fed on virus horse 12, which had been infected by the injection of mosquitoes after an interval of one week. On virus horse 13, 20 more specimens engorged themselves. The mosquitoes were caught partly as adults, partly reared from larvae or pupae. They survived better than those of *A. caballus* and after two weeks 49 specimens or nearly 50 per cent. were still alive, of which 34 fed again.

On the other virus horses which were used in the preceding experiments with *A. caballus*, no *A. lineatopennis* could be fed as at that time no more specimens of this species, adults or larvae, could be obtained.

Virus horses 12 and 13 were used and mosquito groups:—

Group 21*a*. -Fed on virus horse 12 during the night of 21st to 22nd March, 1932. Second day of fever. Temperature 103·6-103·6°. 24 specimens engorged (caught as adults). Used for experiment 29.

Group 22.—Fed together with group 21a. 27 specimens engorged (reared from larvae). Used for experiment 29.

Group 23.—Fed on the same virus horse during the following night. Third day of fever. Temperature 106·2-104·5°. 38 specimens engorged (reared from larvae). Used for experiment 29.

Group 24. Fed on virus horse 13 during the night of 24th-25th March. Third day of fever. 20 specimens engorged (caught as adults). Used for experiment 29.

Experiment 29 (*H.S.* 29). 42 *Aëdes lineatopennis. Feeding. Interval* 16-22 *days. Horse* 20315.

On 6th April, 1932, *A. lineatopennis* groups 21A and 22 were put on to this horse and 22 specimens (out of 25) fed during the following night. Group 23 was fed on the same horse during the night of 8th to 9th April, and 9 specimens (out of 21) engorged themselves. Group 24 was fed during the night of 11th-12th April, and 3 specimens took up blood. Finally, the combined groups 21A-23 were again put on to the horse on the 12th, and 8 specimens fed during the night.

The horse was, therefore, bitten 42 times in all by:—

22 specimens (groups 21A-22) after an interval of 16 days.
9 specimens (group 23) after an interval of 17 days.
3 specimens (group 24) after an interval of 18 days.
8 specimens (groups 21A-23) after an interval of 21-22 days.

These mosquitoes had fed on the infected horse during the second and third days of fever.

Reaction: A brief elevation of the temperature up to 103° occurred on 9th April, one day after the feeding of the second group of mosquitoes, but it lasted only half a day. During an observation period of 48 days, up to 27th May, no real temperature reaction occurred, only 6 times was 101° reached or slightly exceeded, but on each occasion for not longer than 12 or 24 hours.

Immunity test: On 27th May the horse was injected subcutaneously with 1 c.c. blood of horse 20302 (virus horse 11). Five days later the temperature began to rise. On 1st June it was 103·8 and 105°; the 2nd, 105 and 105°; and the 3rd, 103·6 and 100·4°. The following night the horse died, the post-mortem showing typical signs of horsesickness.

Result: The experiment was *negative*, no temperature reaction occurring after the feeding of the mosquitoes, and the horse was normally susceptible to the subcutaneous injection of 1 c.c. virus blood.

F. Experiments with *Aëdes hirsutus*.

At the commencement of our experiments with the Eshowe strain we had at our disposal quite a large supply of *Aedes hirsutus* from one of our breeding places. We succeeded in feeding 311 specimens on the first virus horse injected with the original material of this strain. The mortality amongst these mosquitoes was very low. After a week 100 mosquitoes were injected and after 20-21 days there were still 107 of the remaining specimens alive.

Two experiments were made with these mosquitoes. In the first, experiment 30, 100 specimens were injected after an interval of one week. This experiment was run in conjunction with experiment 24 in which combined groups of *Aëdes caballus* and *A. lineatopennis* were injected after the same interval. The two experiments were intended to serve as controls to one another. In the second experiment, No. 31, the mosquitoes were fed, in the same manner as in the experiments with *A. caballus* and *A. lineatopennis*, on a susceptible horse, and the remaining specimens were injected into the horse of experiment 30 after an interval of nearly one month.

Only one virus horse, No. 11, was used, and mosquito groups:—

Group 10.—Fed on virus horse 11 during the night of 1st to 2nd March, 1932. First day of fever. Temperature 103·0-102·0°. 201 specimens engorged (reared from larvae). Used for experiments 30 and 31.

Group 11.—Fed on the same virus horse the following night. Second day of fever. Temperature 104·8-103·2°. 110 specimens engorged (reared from larvae). Used for experiments 30 and 31.

Experiment 30 (*H.S.* 19). 146 *Aëdes hirsutus. Injection. Interval* 7-30 *days. Horse* 20280.

The horse of this experiment had previously been used in December, 1931, for experiment 13 (O-virus, *A. dentatus*, injection interval 16 days), but no reaction had followed the injection of these mosquitoes.

On 9th March, 1932, 50 *Aëdes hirsutus* group 10 were injected and the following day 50 specimens of group 11. After this the remaining specimens of both groups were used in the following experiment for feeding. Of these, 46 specimens were still alive on 31st March and injected on that date into the horse of this experiment.

Altogether 146 specimens were injected into the horse:—

100 specimens after an interval of 7 days.
46 specimens after an interval of 20-30 days.

These mosquitoes had fed on the virus horse during the first and second days of fever.

Reaction: Directly after the injection of the second lot of mosquitoes a slight rise in temperature occurred (101·4°), and 10 days later another, up to 102°, lasting, however, only half a day. After the third injection the temperature rose to 102°, but by the next morning it had already commenced falling again. The mosquito injections were, therefore, tolerated very well. Up to 27th May, 57 days after the last and 79 days after the first injection of mosquitoes, no fever reaction had occurred, the highest temperature recorded, except for the days mentioned above, being 101°.

Immunity test: On 27th May, the horse was injected subcutaneously with 1 c.c. blood of horse 20302 (virus horse 11). Five days later, on 1st June, the temperature rose to 104·4 and 105°, remaining the following day at 105°. On 3rd June, it was 103·8 and 105°, and the following morning 104·2°. The same day the horse died from horsesickness.

The *result* of the experiment was absolutely *negative*. After the injection of 146 mosquitoes, fed 7-30 days before on an infected horse, no temperature reaction at all followed, whereas the horse proved to be fully susceptible when injected subcutaneously with 1 c.c. of the same strain. It may be remembered that with *Aëdes caballus* and *A. lineatopennis* under the same conditions a positive result had been obtained.

Experiment 31 (*H.S.* 21). *Aëdes hirsutus. Feeding. Interval* 15-21 *days. Horse* 20130.

During the night of 16th to 17th March, 1932, *Aëdes hirsutus* group 10 was allowed to feed on this horse, and out of 114 specimens 72 took up blood. During the following night group 11 was put on to the horse and 19 (out of 43) specimens engorged themselves. Both groups were put on to the same horse again during the night of 22nd-23rd March, and 96 specimens (out of 107) fed. On 31st March, 9 days later, the remaining 46 specimens of these two groups were injected into horse 20280, as described in the preceding experiment.

The horse of this experiment was bitten 187 times by the mosquitoes, viz.:—

91 specimens after an interval of 15 days.
96 specimens after an interval of 20-21 days.

These mosquitoes had fed on the virus horse during the first and second days of the fever.

Reaction: The first afternoon, when the mosquitoes were put on to the horse, the temperature rose to 101°. This was a frequent occurrence when the horses would not willingly enter our mosquito stables and force had to be used. After this, the temperature remained between 98·8 and 100·7°, thus normal up to 5th April, i.e. 20 days after the first and 14 days after the last feeding of mosquitoes. A short fever reaction then set in, the following temperatures being registered: 6th April, 99·4 and 101·9°; the 7th, 99 and 102·8°; the 8th, 99·2 and 103·8°; the 9th, 99·4 and 101·5°. After this the temperature dropped to normal and remained so for a further observation period of 18 days, during which period 100·4° was the highest temperature noted.

Subinoculation: On 8th April, during the short febrile reaction, 5 c.c. blood of horse 20130 was injected into horse 20271.

This horse had previously been used in December, 1931, in experiment 12 (O-virus, *Aëdes dentatus*, injection, interval 5 days), and afterwards in February, 1932, in experiment 18 (O-virus after mosquito passage, *Aëdes* spp., injection after 8-9 days). In both experiments no reaction resembling horsesickness fever followed the injection of the mosquitoes.

The horse was kept under observation for 35 days, up to 13th April. The day after the injection the temperature rose up to 101°, but returned to normal the following day and remained so until the end of the experiment, 100·2° being the highest temperature registered.

Immunity test: On 28th April, 42 days after the first, 37 days after the last feeding of the mosquitoes, and 19 days after the end of the short febrile reaction, the horse (20130) of the main experiment was injected subcutaneously with 1 c.c. blood of horse 20302 (virus horse 11).

The temperature remained normal for three days. On 2nd May, the fourth day p.i., it was 100·5 and 103·6°; the 3rd, 101·4 and 105°; the 4th, 103·8 and 106°; the 5th (a.m.), 104·4; and the 6th (a.m.), 102°. During the same day the horse died. The post-mortem confirmed the diagnosis of horsesickness.

Result: The original horse, on which the mosquitoes had fed, showed a brief febrile reaction of a remittent character, lasting 4 days and showing 103·8° as maximum temperature. This reaction occurred 14-20 days after the feeding of the mosquitoes. Tested with the same virus 19 days later, the horse proved to be normally susceptible. A subinoculation of blood taken during the fever period and injected into another horse, failed to give any reaction. The febrile reaction in the first horse was, therefore, not due to horsesickness fever, and the whole experiment has to be regarded as *negative*.

G. EXPERIMENTS WITH *Aëdes dentatus*.

Aëdes dentatus were not present in large numbers amongst our material during these experiments, nor were they actually rare. In all, 129 specimens could be fed on the five virus horses injected with the Eshowe strain, 21 on virus horse 11, 73 on horse 12, 7 on horse 13, 9 on horse 14 and 18 on horse 15. Of these mosquitoes at least 52 specimens could be induced to feed 67 times on the susceptible horse. The result was, therefore, quite satisfactory.

The virus horses 11 to 15 were used and mosquito groups:—

Group 9.—Fed on virus horse 11 during the night of 2nd to 3rd March, 1932. Second day of fever. Temperature 103·0-102·0°. 13 specimens engorged (reared from larvae). Used for experiment 32.

Group 10.—Fed on the same horse the following night. Third day of fever. Temperature 105·5-103·9°. 8 specimens engorged (reared from larvae). Used for experiment 32.

Group 14.—Fed on virus horse 12 during the night of 21st to 22nd March. Second day of fever. Temperature 103·6-103·6°. 6 specimens engorged (caught as adults). Used for experiment 32.

Group 15.—Fed on the same horse together with group 14. 67 specimens engorged (reared from larvae). Used for experiment 32.

Group 16.—Fed on virus horse 13 during the night of 24th to 25th March. Third day of fever. 7 specimens engorged (caught as adults). Used for experiment 32.

Group 18.—Fed on virus horse 14 during the night of 19th to 20th April. First day of fever. Temperature 103·2 and 101°. 9 specimens engorged (reared from larvae). Used for experiment 32.

Group 19.—Fed on virus horse 15 during the night of 26th to 27th April. Fourth day of fever. Temperature 104 and 106°. 18 specimens engorged (reared from larvae). Used for experiment 32.

Experiment 32 (*H.S.* 23). *Aëdes dentatus. Feeding: Interval* 15-22 *days. Horse* 20291..

This horse had been used before in December, 1931, in experiment 8 (O-virus, *Aëdes caballus*, injection, interval 15-16 days). No typical reaction had followed the injection of the mosquitoes.

On 23rd March, 1932, the mosquito groups 9 and 10 were put on to this horse and during the following night 4 (out of 15) specimens engorged themselves. During the night of 6th-7th April the groups 14 and 15 were allowed to feed and 36 (out of 37) specimens took up blood. On 11th April group 16 was put on to the horse resulting in the feeding of 3 specimens during the ensuing night. Groups 14 and 15 were again put on to the same horse during the night of 12th-13th April and 15 specimens engorged themselves. During the night of 10th-11th May 4 specimens of group 18 were fed and lastly, during the following night 5 of group 19.

Altogether the horse was bitten 67 times by these mosquitoes, viz.:—

5 specimens (group 19) after an interval of 15 days.
36 specimens (group 14-15) after an interval of 16 days.
3 specimens (group 16) after an interval of 18 days.
4 specimens (group 9-10) after an interval of 20-21 days.
4 specimens (group 18) after an interval of 21 days.
15 specimens (group 14-15) after an interval of 22 days.

These different mosquitoes had fed on four different virus horses during the first to third days of fever.

On 28th April and 6th May *Aëdes dentatus* infected with the Kaalplaas virus were fed on the same horse. This will be referred to under experiment 35.

Reaction: The horse was kept under observation up to 3rd June, 72 days after the feeding of the first batch of mosquitoes and 23 days after the last group. During this period no fever reaction occurred, the highest temperature registered being 101·2°.

Immunity test: On 3rd June the horse was injected with 1 c.c. blood of horse 20302 (virus horse 11) subcutaneously. Five days later, on 8th June, the temperature commenced rising and 102° was recorded. On the 9th it was 102·4 and 104°: the 10th, uncertain (anus open, intravenous injection of ·002 gm. Akiron per Kg., body-weight); the 11th, 103 and 104°; and the 12th (a.m.), 104°. The same day the animal died from horsesickness (dunkop).

Result: The experiment has to be regarded as *negative* as no temperature reaction was shown after the application of the mosquitoes, whereas the horse proved to be normally susceptible when injected subcutaneously with material of the same strain.

H. Discussion of the Results with the Eshowe Virus.

A fresh virus strain obtained from a mule previously immunized against O-virus at Eshowe was designated Eshowe virus. Its relationship to O-virus was thus the same as was the case with the Losperfontein strain, and it was, therefore, not the type of strain we really wanted.

With the original material we injected one horse and with its blood two other animals. Another horse was injected after the virus had been subjected to cataphorization by the passage of an electrical current of 9 milliamperes at 250 volts for 4 hours, and a fourth by the injection of mosquitoes. Mosquitoes were fed on all these horses for our experiments.

Altogether 1,650 mosquitoes were fed on the virus horses, 1,068 *Aëdes caballus*, 272 *A. lineatopennis*, 211 *A. hirsutus* and 129 *A. dentatus*. The mosquitoes were caught partly as adults in the fields, partly reared in the laboratory from larvae. Of *A. hirsutus* only specimens reared in captivity were used. During the experiments the mosquitoes were kept in jars or cages in the warm room of the laboratory, in which the temperature varied between 20°, 30° C. with a probable mean of 24°. As a rule, during these experiments, the room was only heated during the day-time, as there was no automatic control. At night the room was actually warmer than out of doors, whereas during the day-time it was cooler. As usual, between the two feedings on the infected and normal horses, the mosquitoes were fed on 10 per cent. sugar water, renewed every day or every second day.

In this series of experiments the mosquitoes were partly injected and partly fed on the susceptible horses. We will discuss first of all the two experiments by means of mosquito injection.

In the first experiment (No. 24) 68 *Aëdes caballus* and 66 *A. lineatopennis*, thus 134 specimens, were injected into a horse in the form of an emulsion on three consecutive days. In this horse a fever reaction made its appearance 8 days after the last, and 10 days after the first injection. Four days later the animal died from horsesickness. All these mosquitoes had fed on an experimentally infected horse 7 days before being injected. In one or more specimens the virus must, therefore, have been present after this lapse of time.

In the second experiment (No. 30), *Aëdes hirsutus* were used. They had fed together with *A. caballus* and *A. lineatopennis* of the preceding experiment. Seven days after their feed on an infected horse 100 specimens were injected in the form of an emulsion on two consecutive days, and 46 more specimens of the same groups after an interval of 29-30 days. No temperature reaction whatsoever followed these injections however, and later the horse proved to be normally susceptible when injected subcutaneously with material from the same strain.

In these two experiments, therefore, virus could be demonstrated by means of the injection of mosquitoes in a combined lot of 134 *A. caballus* and *A. lineatopennis* after an interval of 7 days, whereas in 100 *A. hirsutus* after 7 days and in 46 after nearly a month no virus was present.

By feeding the mosquitoes the following experiments were carried out:—

Aëdes caballus (experiment 25). Interval 16-17 days: 10 specimens. Result, negative.

Aëdes caballus (experiment 26). Interval 18 days: 4 specimens. Interval 20 days: 14 specimens. Total, 18 specimens. Result, negative.

Aëdes caballus (experiment 27). Interval 15 days: 3 specimens. Result, negative.

Aëdes lineatopennis (experiment 29). Interval 16-18 days: 34 specimens. Interval 21-22 days: 8 specimens. Total 42 specimens. Result, negative.

Aëdes hirsutus (experiment 31). Interval 15 days: 91 specimens. Interval 20-21 days: 96 specimens. Total 187 specimens. Result, negative.

Aëdes dentatus (experiment 32). Interval 15-18 days: 44 specimens. Interval 20-22 days: 23 specimens. Total 67 specimens. Result, negative.

In these 6 experiments together the horses were bitten 327 times by the four *Aëdes* species after the following intervals:—

Interval 15 days ... ... 99 specimens
Interval 16-18 days ... ... 87 specimens.
Interval 20-22 days ... ... 141 specimens.

In none of these experiments in which mosquitoes were fed on a normal horse after an interval of 15 days or longer, did we succeed in transmitting the infection. Four of the six normal horses were tested for immunity after a sufficiently long incubation period, 3 by

subcutaneous injection of 1 c.c. and the other by 5 c.c. virus intra-jugularly and all reacted to horsesickness followed by death. There is every reason to believe that the other horses were normally susceptible as well.

The transmission experiments with this strain were, therefore, also a complete failure.

As was the case with O-virus, this virus may be present in mosquitoes after an interval of one week. In the positive experiment with O-virus *A. caballus*, *A. hirsutus* and *A. lineatopennis* were used, with the Eshowe strain *A. caballus* and *A. lineatopennis;* these two species being common to both experiments. We are unable to say, however, whether this survival of the virus in the mosquitoes up to one week is normal or exceptional. It seems rather to be somewhat exceptional. In any case, the results of the feeding experiments do not permit of our regarding it as the beginning of an actual development of the virus in a suitable insect transmitter.

With regard to the possible reasons for the obvious failure of our transmission experiments we may refer to the discussion of the Losperfontein strain in the preceding chapter. The choice of the mosquito species may have been at fault or the strain of virus may have been the old O-virus, which has perhaps lost its capacity for developing in insects. Other factors, or faults in the experimental technique, cannot have been, in our opinion, of any real importance.

IX. EXPERIMENTS WITH KAALPLAAS VIRUS.

On 13th April, 1932, a horse (No. 20031) showing typical and very advanced symptoms of the dikkop form of horsesickness was brought into the Onderstepoort Laboratories from Kaalplaas, a farm belonging to Onderstepoort and almost contiguous with it. This animal had a temperature of 102·2° and died during the following night. It had been hyperimmunized in September, 1931, of the previous year against horsesickness by means of O-virus.

We were able to feed only one large batch of *A. caballus* and a few *A. dentatus* on this horse before it died. The experimental technique was the same as that adopted in the preceding experiments with the Eshowe strain.

A. Experiments with *Aëdes caballus*.

On account of our having succeeded in feeding a fair number of *A. caballus* on this horse notwithstanding the short time at our disposal between its receipt and death, we decided to perform two experiments by (*a*) injecting a certain number of the mospuitoes after an interval of one week, and (*b*) allowing the remainder to feed on a second horse, commencing at an interval of 15 days.

Mosquito group 30.—Fed on virus horse 16 during the night of 13th-14th April, 1932. Temperature 102·2°. (Day of commencement of fever reaction unknown.) 365 specimens engorged (reared from larvae). Used for experiments 33 and 34.

Experiment 33 (*H.S.* 31). 23 *Aëdes caballus. Injection. Interval* 7 *days. Horse* 20318.

On 21st April, 1932, 25 *A. caballus* (group 25), which had fed 7 days before on a horse suffering from horsesickness, which had contracted the disease spontaneously on the farm Kaalplaas and was then in a moribund condition, were injected subcutaneously after thorough emulsification into horse No. 20318.

Reaction: The horse showed a somewhat irregular temperature, varying normally between 99° and 101°. Eleven days after the injection the temperature rose to 102·2°, remaining elevated, however, for only half a day. Nine days of normal temperature followed, then a rise to 101·2° occurred, and two days later (14th May) 103·4° was recorded, both these elevations, however, lasted less than 24 hours. A further period of normal temperature followed but on 27th May some other mosquitoes were injected and this was followed by a slight rise to just over 101° for a few days. The horse was kept under observation for a further 31 days, but no temperature above 101·2° was registered.

Result: The experiment was *negative* as no temperature reaction of any importance followed the injection of the mosquitoes during an observation period of 67 days. An immunity test was not considered necessary. Another horse from the same lot was tested and found to be fully susceptible.

Experiment 34. *Aëdes caballus. Feeding. Interval* 15-23 *days. Horse* 20323.

On 27th April, 1932, group 30 (*Aëdes caballus*) was put on to this horse and during the following night 75 specimens engorged themselves. The remaining specimens of this group were fed on the same horse on the night of 6th to 7th May, and on this occasion 7 specimens took up blood.

The horse was therefore bitten 82 times by these mosquitoes, after an interval of 15 days by 75 specimens, and after an interval of 25 days by 7 specimens. The mosquitoes had been fed on a spontaneous case of horsesickness shortly before the death of the animal.

Reaction: No temperature reaction at all followed the feeding of the mosquitoes, during an observation period of 36 days, the highest temperature registered during this period being 100·8°.

Immunity test: On 3rd June, 36 days after the first and 29 days after the last feeding of the mosquitoes, the horse was injected subcutaneously with 1 c.c. preserved blood of horse 20031, the virus horse used in these experiments. On 8th June, 5 days after the injection, the temperature rose to 105·2° and remained between 105 and 106° for three days. The following morning it had fallen to 100° and the horse died the same day, the post-mortem confirming the diagnosis of horsesickness.

Result: This experiment was clearly *negative.* No temperature reaction had followed the feeding of 82 mosquitoes which had taken a feed of infected blood. The horse subsequently proved to be normally susceptible when injected subcutaneously with 1 c.c. preserved blood of the same strain.

B. Experiment with *Aëdes dentatus.*

On the afternoon on which we received the infected horse from Kaalplaas we had at our disposal only a few mosquitoes of this species, of which 14 only could be induced to feed.

Mosquito group 17.—Fed on virus horse 16 during the night of 13th-14th April, 1932. Temperature 102·2°. Day of commencement of fever reaction unknown. 14 specimens engorged (reared from larvae). Used for experiment 35.

Experiment 35 (*H.S.* 23). *Aëdes dentatus. Feeding. Interval* 15-23 *days. Horse* 20291.

This horse had been used previously, in December, 1931, in experiment 8 (O-virus, *A.caballus*, injection, interval 15-16 days), which had been negative.

From 23rd March, 1932, until the end of this experiment it was used also for experiment 32 (Eshowe virus, *A. dentatus*, feeding).

On 28th April, *Aëdes dentatus* group 17 was put on to this horse and during the following night 5 (out of 8) specimens engorged. The remaining mosquitoes were fed on the same horse again during the night of 6th-7th May and 4 (out of 6) specimens fed on this occasion. The mosquitoes, therefore, fed 9 times on this horse as follows:—

5 specimens after an interval of 15 days.
4 specimens after an interval of 23 days.

Reaction: The horse was kept under observation up to 3rd June, without any reaction being noticed.

Immunity test: The horse was tested for immunity by the subcutaneous injection of Eshowe virus and died 9 days later from horsesickness (*vide* experiment 32).

The *result* of this experiment therefore was *negative*.

C. Discussion of the Results with the Kaalplaas Virus.

About the middle of April, 1932, we received a horse previously hyperimmunized against O-virus, which had contracted horsesickness spontaneously at Kaalplaas. The horse was in a moribund condition when received and had to be made use of for our purpose that same afternoon. One large batch of *Aëdes caballus* and a few *A. dentatus* were fed on this animal before it died. The mosquitoes were kept in our warm room during the experiment under the same conditions as was the case with those of the Eshowe strain.

Three experiments were conducted. In the first (No. 33) 25 *A. caballus* were injected into a horse after an interval of 7 days. No temperature reaction followed this injection so that no virulent virus could have been present.

The remaining specimens of the same group were fed on a second horse (experiment 34), in which 75 specimens engorged themselves after an interval of 15 days and 7 specimens after 23 days. No temperature reaction followed, while the horse proved to be susceptible when inoculated later with the same strain. This experiment was also negative.

Only one experiment was conducted with *Aëdes dentatus* 5 specimens fed after 15 and 4 after 23 days, without conveying infection to a horse.

No positive results were, therefore, obtained with the Kaalplaas virus. This strain was identical, in certain respects, with those received from Eshowe and Losperfontein. All three were derived from animals previously immunized against horsesickness by O-virus. The possible explanation of the failure to transmit the disease, already advanced in the case of the other two strains, may apply equally well in this case and we therefore refer here to these previous discussions.

X. DISCUSSION OF THE RESULTS OBTAINED IN TRANSMISSION EXPERIMENTS WITH HORSESICKNESS.

During the latter part of the winter of 1931 and the summer 1931-1932 experiments were carried out with the object of finding the natural transmitter of horsesickness, a virus disease of great economic importance in South Africa.

Pitchford (1902) only has carried out actual transmission experiments, and he claims to have transmitted the disease by means of Anophelines. From his account, however, it is not possible to obtain a clear idea as to how his results were obtained and they cannot be regarded as proof that Anophelines are the actual transmitters.

Taking into account the known epidemiological evidence mosquitoes must be regarded as the most probable transmitters. The disease is caused by a virus not transmitted by ordinary contact. In its appearance it is practically limited to the summer months, heavy rainfall being followed by a high percentage of infection. In dry seasons relatively few cases appear ordinarily. The infection is practically only transmitted during the night or near sunset or sunrise, and stables afford a relatively good protection. This evidence is sufficient to allow of horsesickness being regarded as an insect-borne disease. However, it is in itself not sufficient to enable the potential transmitters to be narrowed down to a certain group of insects with certainty, although, assuming the epidemiological facts as being correct, mosquitoes must then be regarded as the most probable transmitters. At any rate, none of the facts mentioned oppose the theory of mosquitoes being the natural transmitters, and on this theory we have based our work.

Together with these experiments a mosquito survey was carried out at Onderstepoort, covering the latter part of the winter of 1931, and the whole summer 1931-1932. Mosquitoes were caught in traps containing horses as bait animals, and as thorough a search as possible was made for breeding places. The season at our disposal was very dry and all the information desired could, therefore, not be gained. A review of the results of this survey has already been given in the second paper of this series in which we arrived at the conclusion that certain *Aëdes* species accurately fulfilled the requirements of the potential carriers as laid down by the known epidemiological evidence already mentioned. Amongst the species of this genus, *Aëdes caballus*, *A. lineatopennis*, and *A. hirsutus*, and *Mucidus scatophagoides* must be regarded as the most suitable transmitters, with *Aëdes vittatus* and *A. dentatus* as good potential transmitters, although probably of secondary importance. Owing to lack of experimental information, no definite conclusion could be arrived at regarding the possible rôle of Anophelines.

Our experimental work was based mainly on these conclusions. The majority of the experiments were carried out with the above-mentioned *Aëdes* species. *Mucidus scatophagoides* was discovered to be a blood-sucking species (contrary to the statements in the literature) too late in the season to enable us to carry out experimental work. During the commencement of the work, when no other species were available, a number of experiments were carried out with *Culex theileri*, mainly to obtain technical experience.

Strains of Horsesickness Virus.

For our experiments four different strains of virus were used. Most of the experiments were carried out with the Onderstepoort vaccine strain known as O-virus. This strain had been isolated in 1901 from a spontaneous case of horsesickness. From that date on

it had been transmitted by direct inoculation repeatedly from horse to horse, the 192nd and 225th generations being used in our experiments. Almost invariably the inoculation results in death, the incubation period being usually 2-3 days and the duration of the disease itself generally 3-4 days. For more than 30 years this virus had been transmitted without the normal passage through insects. From the point of view of our work this strain was not very suitable, as, on account of these long years of direct transmissions the developmental capacity of the virus in the insect might have been altered (reduced), thus affecting in particular the value of negative transmission results.

In March, 1932, virus was obtained from a fatal case of horsesickness in a mule at Losperfontein (Transvaal), and from a mule at Eshowe (Natal), both suffering from horsesickness which ended in recovery, and in April from a horse which had contracted a fatal infection at Kaalplaas (Pretoria district). In all these cases a history of immunization against horsesickness with O-virus existed and the possibility cannot be excluded that we have dealt throughout our experiments with O-virus which had appeared with the second attack of horsesickness in the form of a relapse.

Experimental Animals.

The horses used in our experiments were animals of little commercial value, recruited principally from the larger towns in the Transvaal. From the large number of experiments on other phases of horsesickness conducted at Onderstepoort and embodying hundreds of horses it is known that only very exceptionally is an immune horse encountered amongst them.

Experimental Technique.

The experimental technique employed in this work has been discussed in full in the second paper of this series.

The mosquitoes were generally fed on the infected and susceptible horses in small cages enclosed with mosquito netting. In the technique finally adopted these cages were held in place on the backs of the horses by means of a specially constructed saddle which prevented any movement of them even on the more sensitive animals. The mosquitoes were put on to the animals late in the afternoon and removed again the next morning.

Special provision had to be made, at any rate during the summer months, to ensure a sufficiently humid atmosphere for the mosquitoes during their feeding on the horses. In dry surroundings the mortality was extremely high. Very good results were obtained in the specially constructed stable, which was surrounded on all sides by hessian kept wet by a constant flow of water.

In the laboratory the mosquitoes were kept in small jars or in cages consisting of a wooden framework covered with mosquito netting. In these cages a sufficient degree of humidity had also to be provided for. The jars were placed on wet cotton wool in slightly larger jars and the cages on shelves, surrounded on all sides by wet hessian. 10% Sugar solution on cotton wool was provided as food.

The mosquitoes were kept in a room which could be heated electrically. The heating apparatus had to be regulated by hand, however, and variations in the temperature could therefore not be avoided. The average temperature was 24-26° C. but varied from time to time between 20 and 30° C.

Experiments with O-virus.

With O-virus, a strain isolated about 30 years ago, 757 mosquitoes were infected on 9 virus horses, 308 *Culex theileri*, 5 *Anopheles squamosus*, 198 *Aëdes caballus*, 62 *A. lineatopennis*, 43 *A. hirsutus*, 70 *A. dentatus* and 71 *A. vittatus*. Furthermore, between 400 and 500 mosquitoes were fed in one experiment in which the actual number was not ascertained.

With *Culex theileri* the following five experiments were carried out.

Experiment 1. 5 specimens injected after ½ day. Result positive.
Experiment 2. 50 specimens injected after 5 days. Result negative.
Experiment 3. 50 specimens injected after 16 days. Result negative.
Experiment 4. 140 specimens injected after 25 days. Result negative.
Experiment 5. 9 specimens fed, direct transmission. Result negative.

The first experiment, in which 5 *C. theileri* were injected approximately 12 hours after their initial feed, was positive. Sufficient virus was taken up, therefore, by 5 specimens to cause a mortal infection of horsesickness. No conclusions can be drawn from this result as to the ability of *C. theileri* acting as a transmitter, but it indicates that the method of infecting mosquitoes was effective.

In the following three experiments 240 specimens were injected after 5-25 days, 50 of them after 5 days, but in no case was a positive result obtained.

One experiment, by direct transmission with 9 specimens, was also apparently negative. A temperature reaction developed but it could not be traced to horsesickness.

Anopheles squamosus was only used in one experiment.

Experiment 6. 5 specimens injected after 5 days. Result negative.

With *Aëdes caballus* two experiments were carried out.

Experiment 7. 94 specimens injected after 5 days. Result negative.
Experiment 8. 28 specimens injected after 15-16 days. Result negative.

Both experiments, in which 122 specimens were injected, proved to be negative.

Aëdes lineatopennis was also used in two experiments.

Experiment 9. 5 specimens injected after 5 days. Result negative.
Experiment 10. 25 specimens injected after 5 days. Result negative.

In 30 specimens no virus could be detected, therefore, after an interval of 5 days.

Aëdes hirsutus was only used in one experiment, which was negative.

Experiment 11. 30 specimens injected after 5 days. Result negative.

With *A. dentatus* two experiments were made.

Experiment 12. 6 specimens injected after 5 days. Result negative.
Experiment 13. 12 specimens injected after 16 days. Result negative.

In 18 specimens no virus was present after 5-16 days.

With *A. vittatus* two experiments were also carried out.

Experiment 14. 20 specimens injected after 5 days. Result negative.
Experiment 15. 32 specimens injected after 15 days. Result negative.

Both experiments with 52 specimens were negative.

Finally, an experiment was carried out in which a large number, 294 mosquitoes, consisting of 85 *A. caballus*, 115 *A. hirsutus*, and 94 *A. lineatopennis* were injected together after an interval of 6 days. This experiment was *positive* and the result could be confirmed by subinoculation.

With *Aëdes* species 10 experiments were carried out. Only one experiment was positive, in which almost 300 specimens belonging to three different species were injected after an interval of 6 days. In the remaining 9 negative experiments 94 *A. caballus* were injected after 5 days and 28 after 15 days, 6 *A. dentatus* after 5 and 12 after 16 days, 30 *A. hirsutus* after 5 days, 30 *A. lineatopennis* after 5 days and 20 *A. vittatus* after 5 and 32 after 15 days. In all, 180 specimens were injected after 5 and 72 after 15 days.

Experiments with O-virus after one short passage through mosquitoes.

In the preceding series of experiments one positive result was obtained by injecting almost 300 specimens of *Aëdes* 6 days after their having fed on an infected horse. We hoped that the virus had adapted itself to the development in mosquitoes and therefore used this strain in the following two experiments.

Experiment 17. 28 *A. caballus* injected after 7-8 days. Result negative.
2 *A. hirsutus* injected after 7-8 days. Result negative.
27 *A. lineatopennis* injected after 7-8 days. Result negative.

Experiment 18. 157 *A caballus* injected after 8-9 days. Result negative.
94 *A. lineatopennis* injected after 8-9 days. Result negative.
35 *A. hirsutus* injected after 8-9 days. Result negative.
11 *A. dentatus* injected after 8-9 days. Result negative.
2 *A. punctothoracis* injected after 8-9 days. Result negative.
1 *A. cumminsi* injected after 8-9 days. Result negative.

Both experiments were negative. Altogether 357 specimens had been injected after an interval of 7-9 days, 185 *A. caballus*, 121 *A. lineatopennis*, 37 *A. hirsutus*, 11 *A. dentatus* and three specimens of two other species.

There was no sign that the developmental capacity of the virus in the *Aëdes* species used had increased by the short passage in mosquitoes, and further work with this strain was abandoned.

Experiments with Losperfontein Virus.

With a strain of virus derived from a mule, which had been immunised with O-virus some months before, five experiments were conducted. One virus horse was used and on it 1,035 mosquitoes were fed, 816 *A. caballus*, 151 *A. lineatopennis*, 61 *A. dentatus* and 7 *A. vittatus*.

With *A. caballus* the following experiment was carried out:—

Experiment 19. 71 specimens fed after 14-15 days. Result negative.
6 specimens fed after 20 days. Result negative.

77 specimens, refed after 14-20 days, failed to transmit the disease.

A. lineatopennis was made use of in two experiments:—

Experiment 20. 38 specimens refed after 13-17 days. Result negative
51 specimens refed after 19-21 days. Result negative.
66 specimens refed after 33-35 days. Result negative.
10 specimens refed after 60-62 days. Result negative.
Experiment 21. 6 specimens injected after 63-65 days. Result negative.

Both experiments were negative. In the first the horse was bitten 165 times by batches of *A. lineatopennis* infected 13-62 days previously. In the remaining six specimens of the same batches no virus could be traced by injection after 63-65 days.

With *A. dentatus* the following experiment was made:—

Experiment 22. 20 specimens refed after 25-27 days. Result negative.
6 specimens refed after 35-37 days. Result negative.

A. vittatus was made use of in the last experiment:—

Experiment 23. 5 specimens refed after 23 days. Result negative.
4 specimens refed after 37 days. Result negative.

Altogether four horses were bitten 277 times by specimens of *A. caballus*, *A. lineatopennis*, *A. dentatus* and *A. vittatus* as follows: After 13-17 days by 109 specimens, after 19-21 by 57, after 23-27 by 58, after 33-37 by 43 and after 60-62 by 10 specimens. Furthermore, six specimens were injected after 63-65 days. In none of these experiments were positive results or reactions, which could be suspected of being horsesickness fever, obtained.

Experiments with Eshowe Virus.

Towards the end of February another strain was received, isolated from a spontaneous case of horsesickness in a mule, which, however, had also been immunised previously with O-virus.

In all, four virus horses were used in which 1,680 mosquitoes were fed, 1,068 *A. caballus*, 272 *A. lineatopennis*, 211 *A. hirsutus* and 129 *A. dentatus*.

In the first experiment a number of *A. caballus* and *A. lineatopennis* were injected together a week after their having fed on a virus horse.

Experiment 24. 68 *A. caballus* injected after 7 days. Result positive.
66 *A. lineatopennis* injected after 7 days. Result positive.

The temperature of the horse commenced to rise 9 days after the first and 7 days after the last injection. The disease itself, which ended in death, lasted 6 days. The diagnosis of horsesickness was confirmed on post-mortem.

With *A. caballus* alone three experiments were made:—

Experiment 25. 10 specimens refed after 16-17 days. Result negative.
Experiment 26. 18 specimens refed after 18-20 days. Result negative.
Experiment 27. 3 specimens refed after 15 days. Result negative.

In all, 31 specimens fed after 15-20 days without transmitting the infection.

A. lineatopennis was used in the following experiment, which also was negative:—

Experiment 29. 34 specimens refed after 16-18 days. Result negative.
8 specimens refed after 21-22 days. Result negative.

With *A. hirsutus* the following two experiments were carried out:—

Experiment 30. 100 specimens injected after 7 days. Result negative.
46 specimens injected after 29-30 days. Result negative.
Experiment 31. 91 specimens refed after 15 days. Result negative.
96 specimens refed after 20-21 days. Result negative.

Both experiments were negative. The mosquitoes from the second experiment which were still alive after a month, were used for the second injection in the first experiment. In the second experiment a short febrile reaction appeared 14-20 days after the injection of the mosquitoes. The horse, however, later proved to be normally susceptible and a subinoculation of blood, taken during the febrile reaction, failed to infect another horse.

In the last experiment *A. dentatus* was used, also with negative results:—

Experiment 32. 44 specimens refed after 15-18 days. Result negative.
23 specimens refed after 20-22 days. Result negative.

The first experiment with this strain, in which a combined lot of 134 *A. caballus* and *A. lineatopennis* was injected gave a positive result. We were therefore quite hopeful as to the suitability of this strain. All subsequent experiments were however, negative.

In these negative experiments 146 *A. hirsutus* were injected after 7-30 days and 31 *A. caballus* refed after 15-20 days, 42 *A. lineatopennis* after 16-22 days, 187 *A. hirsutus* after 15-21 days and 67 *A. dentatus* after 15-22 days. In all the horses were bitten by 327 specimens of these four species after intervals ranging between 7 and 30 days.

Experiments with Kaalplaats Virus.

In April, 1932, a horse suffering from horsesickness which had previously been hyperimmunised against O-virus, was received from the farm Kaalplaats. It died the following morning, but in the interim between its receipt and death we succeeded in feeding 365 *A. caballus* and 14 *A .dentatus* on it.

With *A. caballus* two experiments were carried out, which yielded negative results:—

Experiment 33. 25 specimens injected after 7 days. Result negative.
Experiment 34. 75 specimens refed after 15 days. Result negative.
7 specimens refed after 23 days. Result negative.

In the last experiment *A. dentatus* were fed, also without any result:—

Experiment 35. 5 specimens refed after 15 days. Result negative.
4 specimens refed after 23 days. Result negative.

In all, 25 *A. caballus*, which had fed on a spontaneous case of horsesickness, were injected after 7 days, 82 specimens of the same species refed after 15-23 days and 9 *A. dentatus* refed after 15-23 days. In none of the experiments was a positive result obtained.

General Discussion of the Results.

On horses infected with four different strains of horsesickness, the O-virus vaccine strain and one strain each from Losperfontein, Eshowe and Kaalplaas, 4,254 mosquitoes were fed, 308 *Culex theileri*, 5 *Anopheles squamosus*, 2,655 *Aëdes caballus*, 628 *A. lineatopennis*. 292 *A. hirsutus*, 285 *A. dentatus*, 78 *A. vittatus*, 2 *A. punctothoracis* and 1 *A. cumminsi*. Furthermore, in one experiment, in which the actual number was not ascertained, at least 400-500 specimens had fed. To obtain these results, several times this number of mosquitoes, at a rough estimation, 10,000 specimens, had to be collected and handled.

With these mosquitoes 34 experiments were carried out in all (excluding experiment 28, in which the mosquitoes fed during a fever reaction, which, at first, was regarded as horsesickness fever, a diagnosis which, however, was not confirmed by the further experiments). In these experiments 1,434 mosquitoes were injected after ½-65 days, viz.; 245 *Culex theileri*, 5 *Anopheles squamosus*, 485 *Aëdes caballus*, 287 *A. lineatopennis*, 328 *A. hirsutus*, 29 *A. dentatus*, 52 *A. vittatus*, 2 *A. punctothoracis* and 1 *A. cumminsi*. A large number of specimens was refed on susceptible horses at intervals varying from about 1 minute to 62 days, 704 feedings actually taking place, viz.: 9 feedings with *Culex theileri*, 190 with *Aëdes caballus*, 207 with *A. lineatopennis*, 187 with *A. hirsutus*, 102 with *A. dentatus* and 9 with *A. vittatus*.

Positive results were obtained only by injections of mosquitoes. In the first case (experiment 1), five *Culex theileri* were injected the morning after having fed on an infected horse. This result only demonstrated, however, that sufficient virus to produce an infection had been taken up by 5 mosquitoes. In the second experiment (No. 16), 294 mosquitoes, 85 *A. caballus*, 115 *A. hirsutus and* 94 *A. lineatopennis*, which had fed 6 days before on a horse infected with O-virus, were injected. In the third positive case (experiment 24), 68 *A. caballus* and 66 *A. lineatopennis* had been injected. These mosquitoes had fed 7 days previously on a horse infected with Eshowe virus.

The horsesickness virus can therefore retain its full virulence in Aëdes species up to 7 days. This result was obtained with two strains of virus, and *A. caballus*, *A. lineatopennis* and *A. hirsutus* were the species concerned, the former two species being common to both experiments.

The remaining 31 experiments were negative. We will combine the experiments made with the different virus strains according to the species used.

With *Culex theileri* four experiments were carried out with O-virus (Nos. 2-5). 240 specimens were injected after 5-25 days, viz.: 50 after 5, 50 after 16 and 140 after 25 days. Direct transmission was also attempted by partially feeding 9 specimens on an infected horse and immediately thereafter on a susceptible horse.

With *Anopheles squamosus* one experiment was conducted with O-virus in which 5 specimens were injected after 5 days (experiment 6).

Aëdes caballus was used in 10 experiments, four with O-virus (experiments 7-8, 17-18), one with Losperfontein virus (experiment 19), three with Eshowe virus (experiments 25-27) and two with Kaalplaas virus (experiments 33-34). 332 specimens were injected after 5-16 days, viz., 94 after 5, 210 after 7-9 and 28 after 15-16 days. Mosquitoes were refed 190 times on susceptible horses after 14-23 days, viz., 159 after 14-17, 24 after 18-20 and 7 after 23 days.

Aëdes lineatopennis was used in 7 experiments, four with O-virus (experiments 9-10, 17-18), two with Losperfontein virus (experiments 20-21) and one with Eshowe virus (experiment 29). 157 specimens were injected after 5-65 days, viz., 30 after 5, 121 after 7-9 and 6 after 63-65 days. 207 specimens were refed on susceptible horses as follows:—72 after 13-18, 59 after 19-22, 66 after 33-35 and 10 after 60-62 days.

Aëdes hirsutus were used in five experiments, three with O-virus (experiments 11 and 17-18) and two with Eshowe virus (experiments 30-31). 213 specimens were injected after 5-30 days, viz., 30 after 5, 137 after 7-9 and 46 after 29-30 days. 187 specimens refed after 15-21 days, viz.: 91 after 15 and 96 after 20-21 days.

Aëdes dentatus was used in 5 experiments, three with O-virus (experiments 12, 13 and 18), one with Losperfontein virus (experiment 32) and one with Kaalplaas virus (experiment 35). 29 specimens were injected after 5-16 days, viz., 6 after 5, 11 after 8-9 and 12 after 15 days. 102 specimens refed after 15-37 days, viz.: 49 after 15-18, 27 after 20-23, 20 after 25-27 and 6 after 35-37 days.

Aëdes vittatus was used in three experiments, two with O-virus (experiments 14-15) and one with Losperfontein virus (experiment 23). 53 specimens were injected after 5-13 days, viz.: 20 after 5 and 32 after 15 days. 9 specimens refed after 23-37 days, viz.: 5 after 23 and 4 after 37 days.

Aëdes punctothoracis and *A. cumminsi* were used in the experiment with O-virus (experiment 18) in which three specimens were injected after 8-9 days.

Amongst the various *Aëdes* species employed the following number of specimens was used:—786 specimens were injected after 5-65 days, viz.: 18 after 5, 482 after 7-9, 72 after 15-16, 46 after 29-30 and 6 after 63-65 days. 695 mosquitoes refed on susceptible horses after 13-62 days, viz.: 375 after 13-18 days, 214 after 9-23, 20 after 25-27 days, 76 after 33-57 and 10 after 60-62 days.

Thirty-one experiments, in which (apart from 240 Culex and 5 Anopheles), 786 specimens belonging to Aëdes species were injected after 5-65 days and 695 refed after 13-62 days, were thus negative. We cannot but regard these negative results as significant, in fact even more significant than the few positive results, the more so as these positive results were only obtained by injections of mosquitoes within a short period after their having fed.

Notwithstanding the, undoubtedly, sufficiently large amount of material used, it is not possible to arrive at a definite conclusion as to the results. On several occasions we have laid stress upon the fact

that the strains of virus used in our experiments were not really suitable. Most of the experiments were carried out with O-virus, the laboratory vaccine strain which had been isolated more than 30 years ago and had been transmitted about 200 times from horse to horse by direct inoculation. It must be regarded as possible that this strain, not having been in contact with its invertebrate host for such a long time, has lost its developmental capacity in insects either totally or partially. Furthermore, in each of the strains derived from spontaneous cases a history of immunization with O-virus existed. We therefore might have been dealing throughout the whole course of the experiments with O-virus and if this supposed reduction in its developmental capacity were an actual fact, the value of the negative results would naturally also be reduced.

The best, and perhaps correct, reason for our negative results might be the fact that we were not dealing with the real transmitters. We had this possibility in mind throughout the work. The results of the mosquito survey, based on epidemiological evidence, assumed to be correct but being beyond our control, pointed clearly to certain *Aëdes* species as the most promising transmitters, and, almost daily observations in the field, yielded no further information of importance. It must be remembered, however, that the season at our disposal was not a suitable horsesickness season.

There is, furthermore, the possibility that errors in our methods were responsible for the failures. In the event of the infection index being low, a small number of specimens might be insufficient, but in this case it cannot be an important factor, considering the amount of material we used. The extrinsic incubation period allowed should have been long enough and the temperature at which the mosquitoes were kept was certainly high enough to allow of a rapid development or multiplication of the virus. So far as we can judge there do not appear to exist other factors of any importance which could be regarded as responsible for the failures. Further strong evidence in favour of the view that *Aëdes* species are not the real transmitters is contained in the fact that 662 " infected " specimens belonging to this genus were injected without causing an infection. Horsesickness virus is very resistant and retains its virulence for a number of years at ordinary room temperature nor is it easily destroyed by putrefaction. Yellow Fever virus, on the other hand, which bears a close resemblance to horsesickness virus in many respects, loses its virulence very rapidly at room temperature, yet it remains alive for a considerable time in a large variety of mosquitoes more or less related to its natural transmitter. It appears strange, therefore, that horsesickness virus was so easily destroyed in all the species of mosquitoes used.

Taking every argument into consideration, we must come to the conclusion that *Aëdes species are in all probability not the transmitters of horsesickness. The nature of the strains used, however, makes a definite conclusion not possible.*

In future work, experiments with spontaneous virus strains and *Aëdes* species will, first of all, have to be carried out to ascertain the exact importance of these species, and should these fail, *Anophelines*

will then have to be tested. Furthermore, a thorough mosquito survey, during a suitable season, is of the utmost importance in order to obtain more comprehensive epidemiological data.

SUMMARY.

During the latter part of the summer of 1931-1932 and during the winter of 1932 experiments in connection with the natural transmission of horsesickness were carried out at Onderstepoort. The season was unfavourable on account of a shortage of rain.

The result of a mosquito survey, carried out at the same time and described in the first paper of this series, had pointed out that, taking into consideration the epidemiological evidence generally accepted as correct, certain *Aëdes* species are the most promising transmitters of horsesickness amongst the flying insects. On this assumption mainly was our work based.

Four strains of virus were used, O-virus, the laboratory vaccine strain, and three strains derived from field cases of the disease.

The ordinary experimental technique in mosquito transmission work had to be modified and adapted to the special requirements (horses as experimental animals) and to the South African climatic conditions. The methods used were described in the second paper of this series.

Altogether over 4,500 clean mosquitoes, belonging principally to different species of the genus *Aëdes*, were fed on experimentally infected horses. Over 10,000 specimens had to be caught or reared and handled.

In all, 35 experiments were carried out, in which the mosquitoes were either injected into susceptible horses or refed on them after different intervals.

1,434 specimens were injected ½-65 days, 245 *Culex theileri*, 5 *Anopheles squamosus*, 485 *Aëdes caballus*, 287 *A. lineatopennis*, 328 *A. hirsutus*, 28 *A. dentatus*, 52 *A. vittatus*, 2 *A. punctothoracis* and 1 *A. cumminsi*.

704 mosquitoes were refed at from 1 minute to 62 days after their having fed on infected horses, 9 *Culex theileri*, 190 *A. caballus*, 207 *A. lineatopennis*, 187 *A. hirsutus*, 102 *A. dentatus* and 9 *A. vittatus*.

Three experiments only were positive. In the first of these experiments 5 *Culex theileri* were injected about ½ day after their initial feed, indicating that sufficient virus had been taken up by this number of mosquitoes. In the second positive experiment 85 *A. caballus*, 94 *A. lineatopennis* and 115 *A. hirsutus* were injected after 6 days, and in the third experiment 68 *A. caballus* and 66 *A. lineatopennis* after 7 days. In these mosquitoes it was therefore demonstrated that the virus might remain alive for periods of up to one week.

The remaining experiments were all negative. 240 *Culex theileri* were injected after 5-25 days, 5 *Anopheles squamosus* after 5 days, 332 *Aëdes caballus* after 5-16 days, 157 *A. lineatopennis* after

5-65 days, 213 *A. hirsutus* after 5-30 days, 29 *A. dentatus* after 5-16 days, 52 *A. vittatus* after 5-15 days, 2 *A. punctothoracis* and 1 *A. cumminsi* after 8-9 days.

190 *A. caballus* refed after 14-23 days, 207 *A. lineatopennis* after 13-62 days, 187 *A. hirsutus* after 15-21 days, 102 *A. dentatus* after 15-37 days and 9 *A. vittatus* after 23-37 days.

In all, 786 *Aëdes* species were injected after 5-65 days and 695 *Aëdes* species refed after 13-62 days.

By injections of mosquitoes at intervals of up to 9 days, two positive results were obtained by using 428 specimens, whereas with 662 further specimens only negative results were procured. The virus is usually quickly destroyed, therefore, in the *Aëdes* species, although it is normally very resistant, often remaining virulent at ordinary room temperature for a number of years.

The virus strains themselves were not very suitable for our work. The O-virus strain, with which most of the experiments were carried out, had been isolated about 30 years ago and transmitted through almost 200 generations from horse to horse without any passage through the natural transmitters. We must take into account therefore, the possibility of the virus having lost at least part of its developmental capacity in insects. In the other strains at our disposal, derived from field cases, a history of immunisation with O-virus against horsesickness existed, and it is thus possible that we were dealing throughout our work with O-virus.

From these experiments we arrive at the final conclusion, *that Aëdes species are very probably not the transmitters of horsesickness.* It is, however, impossible to come to a definite conclusion owing to the nature of the strains used.

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Rabies in South Africa.

Occurrence and Distribution of Cases during 1933.

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In comparing the number of positive cases of rabies diagnosed in 1933 with those for the previous year Neitz and Thomas (1932), it will be found that there is a remarkable increase in the incidence of the disease not only in small wild carnivora (*Viverridae*) but also in human beings and domestic animals. The material sent in for laboratory examination is more than double that submitted in 1932. Out of the 48 specimens submitted for laboratory examination from various parts of the Union, 26 proved to be positive for rabies. In our previous report (1932) we were inclined to ascribe this rapid increase in the incidence of rabies more to realisation by the public of the potential danger of viverrids as carriers of rabies and hence more material being sent in for laboratory examination than to a definite spread of the disease. From the observations made during 1933, however, there is little doubt that rabies has spread to an extent that cannot be regarded as other than serious.

It is true that the country has been experiencing more than usually dry seasons lately which culminated this year in one of the worst droughts known. We have little or no knowledge of the habits, food supplies, and other factors affecting the life of our smaller wild carnivora, but it would certainly be very strange if they did not react in some way or other to such unprecedented adverse conditions. It is thus more than probable that migration in search for food must have taken place to a greater or lesser extent and so account for the greater spread of the disease.

It will be interesting in this respect to note the effects of the general and soaking rains which fell after November, 1933. It has been reported that numbers of meercats were drowned in their burrows as a result of the rains. Starvation, disease, possibly cannibalism may have accounted for a considerable additional number. With the return of normal food supplies more stable conditions may again prevail. If the drought has had anything to do with this increased incidence of rabies during the last two years it is probably safe to predict a corresponding decrease in rabies with the return to normal climatic conditions. This should in no way affect the very urgent need for research into the life-history and means of controlling the *Viverridae* in those areas known to be infected.

Discussion of the Cases.

For the sake of convenience the relevant data are tabulated as in our previous report. In Table I will be found all available details of each positive case. Table II gives a summary of the distribution of the disease in the provinces and the species of animal affected. In Table III are given the details of cases examined with negative results.

It will be seen from Table I that whenever suitable material for histo-pathological examination was received Negri bodies were demonstrated with the exception of three cases, namely, 18, 25 and 26. It will be noted, however, that all three these animals were destroyed. The fact that the disease was not allowed to run its course may explain the difficulty or impossibility of finding Negri bodies. This illustrates once again the necessity of controlling by biological inoculation all suspected cases giving negative findings histologically.

The disease in human beings was transmitted once by a dog, twice by the domestic cat, twice by the yellow mungoose, and once by a wild cat (species not determined). The incubation period varied from 3 weeks to 3 months. The mode of transmission to the domestic animals in most cases was not determined, but it can be assumed that one or other member of the family *Viverridae* was responsible.

The incubation period in rabbits used for the biological test varied from 8 to 21 days, usually with an average of 14 days. Cases 1 and 3 show rare exceptions in which the incubation period was 65 and 76 days respectively. Rabbit R. 1013 in Case 1 and Rabbit R. 1415 in Case 25 did not develop rabies in spite of the fact that their mates injected with the same material succumbed to the disease. Two rabbits R. 1362 (Case 23) and R. 1416 (Case 26), showed typical symptoms of the dumb form of rabies, but histologically no Negri bodies were demonstrated.

Case No. 18 is of special interest. The Government Veterinary Officer, Rustenburg, who sent this material from a calf examined by him investigated this outbreak on the farm Leliefontein approximately 60 miles from Rustenburg in the Ventersdorp district. He reports that this farmer had lost 9 calves and three cows over a period of 6 months. These animals were stated to have shown a peculiar gait, in some cases became aggressive, bellowing, frothing from the mouth and exhibiting increased sexual excitability. Later paralysis, coma and then death followed. The symptoms described by the owner of these animals are so typical that there is little doubt that they all contracted rabies and died from it in that period. It appears further that several colonies of meercats were established in close proximity to the kraal in which the cattle in question were kept.

Differential Diagnosis.

The reason for including Table III in the present report is to show as far as possible what other conditions have been encountered and have to be considered in differential diagnosis. Acute forms of

nervous distemper, particularly when accompanied with fits, are of course very commonly and naturally regarded with suspicion. In Case 21 (Table III) the peculiar behaviour of the dog was readily explained when the brain was found to harbour numerous " measles " identified as *Cysticercus cellulosae*.

Incidentally, also it will be noted how often brains useless for histo-pathological examination are submitted. The animals in such cases are evidently killed by stoning or clubbing and the brain and skull unnecessarily battered and pulped as a result, so that it is impossible to find a suitable piece for microscopic preparation.

The Spread of the Disease.

The appended map shows the districts in which rabies is known to have occurred. The remarks made in our previous article regarding the limits of the infection still hold. Comparison of this map with that for 1932 brings out clearly and significantly how districts adjacent to the ones in which rabies has previously been diagnosed are being progressively included in the known infected area. New outbreaks in the Transvaal are: Brakpan, Ventersdorp, and Schweizer Reneke; in the Orange Free State: Fauresmith, Senekal and Edenburg. These newly infected districts are shaded black in the map.

Correction.

Through an oversight the district of De Aar, an old infected area, was not marked accordingly in the map in our previous report for 1932. It is shown correctly shaded in the present one.

Acknowledgments.

Thanks are due to Mr. F. Boughton for his able assistance with the operations and for keeping records, to Mr. C. J. Walker for preparing the map, and to the Head of the Department of Public Health for information supplied.

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TABLE II.

Rabies Diagnosed in.	Transvaal.	Orange Free State.	Cape Province.	Total Number of Cases in the Union.
Humans	1	5	—	6
Dogs	—	1	— (1)	1 (1)
Cats (Domestic)	—	2	2	4
Cattle	1 (11)	—	—	1 (11)
Sheep.	—	2	—	2
Cynictis penicillata	4	11	—	15
Genetta felina.	—	—	1 (1)	1 (1)
Wild Cat (species not mentioned).	—	1	—	1
TOTALS FOR THE YEAR 1933.	6 (11)	22	3 (2)	31 (13)

The figures in brackets refer to clinically positive cases of rabies, material from which was not available for confirmation of diagnoses.

TABLE III.

Month and Year.	Locality. Province.	Locality. District.	Locality. Town or Farm.	Specimen No.	File No.	Material from.	Disease suspected.	Laboratory Examination for Rabies.	Remarks.
Case 1......... January, 1933	O.F.S.	Hoopstad......	Wesselsrequest (Wesselsbron)	11455 11452	141/2474	*Cynictis penicillata*	Rabies.................	—	Skull badly battered. No brain material available for examination.
Case 2......... January, 1933	O.F.S.	Reitz..........	Tweeling.......	12384	141/300	Domestic Cat (Kitten)	Rabies—Aggressive.....	—	Skull badly battered. No brain material available for examination.
Case 3......... February, 1933	O.F.S.	Tromsphurg....	Trompsburg....	13927	141/310	*Cynictis penicillata*	Rabies.................	—	Skull badly battered. No brain material available for examination.
Case 4......... March, 1933	Tvl.	Johannesburg..	Newtown......	15261 15262	126 2	Dog...........	Rabies.................	Negative	Probably a case of nervous distemper.
Case 5......... March, 1933	S.W.A.	Grootfontein...	Abenab Mine..	15439 15540	151/—	Dog (Dobberman bitch)	Rabies: Symptoms: convulsion, swaying, staggering, foaming at the mouth. Bitch apparently recovered, and two months later the same symptoms developed. Animal was shot	Negative	—
Case 6......... April, 1933	Tvl.	Pretoria.......	Pretoria North.	446 447	134/66	Cow...........	Rabies.................	Negative	—
Case 7......... June, 1933	O.F.S.	Senekal........	Resida........	3890	141/303	*Cynictis penicillata*	Rabies.................	—	No brain material available In July owner of farm sent in another *C. penicillata*. See Case No. 17, Table I, which proved to be rabies
Case 8......... June, 1933	C.P.	Grahamstown..	Grahamstown..	3961 3962	144/54	Dog...........	Rabies.................	Negative	Probably a case of nervous distemper.
Case 9......... June, 1933	O.F.S.	Edenburg......	Kromspruit.... (Farm)	4036 4037	141/1146	Dog	Rabies: Three weeks after fighting two sick meercats, dog became stiff, paralysed, and snarled at owner	Negative	—

TABLE III—(*continued*).

Month and Year.	Locality.			Specimen No.	File No.	Material from.	Disease suspected.	Laboratory Examination for Rabies.	Remarks.
	Province.	District.	Town or Farm.						
Case 10........ July, 1933	Tvl.	Pretoria.......	Balmoral......	4329 4330	131/151	Dog...........	Rabies: Two weeks after whelping, bitch developed fits. Death set in 3 days later	Negative	—
Case 11........ August, 1933	Tvl.	Johannesburg..	Modder B Mine	5513 5514	126/451	*Suricata suricatta*	Rabies..	Negative	—
Case 12........ August, 1933	Natal	Richmond.....	Greenhill.......	5690 5691	155/26	Dog...........	Rabies..	Negative	—
Case 13........ Sept., 1933	Tvl.	Ermelo........	Shepstone......	6301 6302	124/12	Dog...........	Rabies..	Negative	Probably a case of nervous distemper.
Case 14........ Sept., 1933	Tvl.	Johannesburg..	Johannesburg..	6428 6429	126/2	*Suricata suricatta*	Rabies..	Negative	—
Case 15........ Oct., 1933	C.P.	Vryburg.......	Illaura Farm...	6753 6754	144/474	Heifer.........	Rabies......	Negative	—
Case 16........ Oct., 1933	C.P.	Vryburg.......	Illaura Farm...	6921 6922	144/474	Bovine...... .	Rabies....... . .	Negative	—
Case 17........ Oct., 1933	O.F.S.	—	Zwartranddam.	7321 7322	144/1964	Horse.........	Rabies.... . ..	Negative	—
Case 18........ Oct., 1933	Tvl.	Potgietersrust..	Klipputgat.....	7324 7325	138/611	Jackal.........	Rabies.. . .	Negative	—
Case 19........ Nov., 1933	O.F.S.	Dealesville.....	Dealesville.....	8132 8133	—	Nagaapie......	Rabies.. .	Negative	—
Case 20........ Nov., 1933	Tvl.	Wolmaransstad.	Klippan farm..	8337 8338	139/71	Dog...........	Rabies	Negative	—
Case 21........ Dec., 1933	Tvl.	Johannesburg..	Braamfontein..	8375 8376	126/2	Dog....... ...	Rabies: Dog found walking about aimlessly on outskirts of town	—	Large number of *Cysticercus cellulosae* were found in the brain.
Case 22........ Dec., 1933	O.F.S.	Trompsburg....	Trompsburg....	9130 9131	141/310	Dog......	Rabies................	Negative	—

Section III.

Parasitology.

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Preliminary Note on the Life-History of *Gaigeria pachyscelis* (Raill. and Henry, 1910), a Hookworm of Sheep.

By

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DURING the last few years investigations have been carried out on the mode of infection, development and methods of prevention and cure of *Gaigeria pachyscelis*, a nematode parasite of sheep which is very prevalent in the drier areas of South Africa. The most important results so far obtained are briefly referred to in this preliminary note; a fuller account of these investigations will be given at a later date.

The material used was obtained in the first place from infected sheep from the Vryburg area of the Union. The sheep were brought to Onderstepoort, and the mature larvae were cultured from their faeces. Ordinary jam bottles were used in which the broken up faeces were placed; they were then incubated at about 26° C., and the mature larvae were collected from the walls of the jars in from 6 to 8 days. This was a relatively simple process as these larvae possess characteristics by which they may easily be distinguished from the larvae of Strongyloides, Wireworms, Trichostrongyles and Oesophagostomes.

INFECTION.

For the purpose of infection sheep were used which had been reared at Onderstepoort where these Hookworms do not occur; they consisted for the most part of lambs less than a year old. Several attempts were made on 18 sheep to bring about oral infection, but in all cases the results were negative. The infective larvae were introduced into the mouths of sheep either in the form of a drench, or in a little water squirted into the sides of the mouth or in a little mash. Meanwhile it had been observed that the infective larvae were not able to withstand drying, and also that they were attracted towards the source of heat, the gentle warmth at the same time being responsible for a very increased activity. Now these responses are very characteristic of those larvae which bring about infection by penetrating intact skin, and attempts at this mode of infection were now made. In the first series of experiments the active larvae, in a little water, were placed between the hoofs of 23 sheep and this

application of larvae was repeated several times during the course of the following weeks. The faeces of all the sheep were regularly examined once a week from the 4th week onwards after the first application of larvae. Positive results, shown by the appearance of the characteristic eggs in the faeces of the sheep, were obtained in practically all the sheep. (20 sheep gave positive results, 1 remained negative, and 2 died shortly after the 1st application of larvae.) The time which lapsed from the time of the 1st cutaneous infection to the first appearance of the eggs in the faeces varied from 10 weeks to 21 weeks and 5 days, the more frequent period, however, being about 10 weeks.

In order to exclude the possibility of the sheep becoming infected through the licking up of active larvae from their hoofs, the hoofs of the exposed sheep in the next series of experiments were enclosed in thick canvas bags, and these bags were kept on the hoofs for at least one day after application of the larvae. Five sheep were used, and these all gave positive results. Unfortunately four sheep died during the 10th week of infection and before any eggs had been seen in the faeces. However, adult Gaigeria were found on post-mortem in the small intestines of all four. The 5th sheep showed Gaigeria eggs in its faeces 11 weeks and 3 days after its first exposure.

Having established that cutaneous infection was possible, the larvae, suspended in a little water, were in the next series of experiments applied to the skin behind the ears. So far this mode of infection has been applied to 34 sheep, and of these 31 have given positive results, either by the finding of the eggs in the faeces or of adolescent or larval stages on post-mortem. One sheep failed to become infected, and of the remaining 2, one died while the writer was away and the other died soon after infection.

Summarising the above-mentioned results we find that of 18 sheep exposed to oral infection none gave positive results, whereas of 62 sheep which had been exposed to cutaneous infection, either between the hoofs or behind the ears, 56 became infected, 2 remained negative, and in the case of 4 no definite results can be given. From these results it appears legitimate to conclude that the normal mode in which sheep become infected is through the skin and not through the mouth.

Development.

The eggs when passed out in the faeces are in the morula stage. With suitable moisture, air and warmth they develop and hatch out in about 24 hours. The larvae feed and grow and undergo their first moult about 24 hours later. These 2nd stage larvae are very similar to those of the preceding stage, and in from 4 to 6 days from the commencement of incubation, depending on the temperature, they reach the 3rd or infective stage. These larvae are ensheathed in the cuticle of the previous stage, which is provided with a long slender tail; they are active but do not feed. They are climbers, are attracted towards the source of gentle heat, cannot withstand drying and can penetrate the intact skin. They vary in length from 0·605 mm. to 0·713 mm. On coming in contact with living skin they become very active, and boring through the skin they

soon reach the lungs presumably via the blood and lymph streams. They remain in the lungs until the 13th or 14th day after entering the skin, and during this period they grow in the lungs and pass into the 4th stage. This stage is characterised by the presence of a globular larval mouth capsule provided with a dorsal tooth and two subventral lancets at its base; sex differentiation has not yet taken place, the genital primordium being still lens-shaped and composed of a few cells; they have increased in length to about 1·4 mm. On the 13th and 14th days after infection the first larvae make their appearance in the small intestine; these are all still in the same stage of development as the 4th stage larvae found in the lungs and show very little if any increase in size. Their course from the lungs to the intestine is probably via the trachea and oesophagus. In the intestine the larvae attach themselves to the villi by drawing the villi into their buccal capsules and lacerating them by means of their teeth. They are reddish in colour and appear to feed on the liberated blood. Growth continues, and sex differentiation is very soon apparent. During the 5th or 6th week after infection they pass into the 5th or final stage: the larval buccal capsule is replaced by the adult buccal capsule and the final ecdysis takes place soon after. From this time onwards the adolescent 5th stage worms gradually grow and become sexually mature in about 10 weeks from the time of their exposure to infection.

No investigations have as yet been carried out in the field, but from the above-mentioned results it is probable that the sheep acquire their infection, in the more arid areas, through their feet from larvae present in the moist earth round about drinking troughs, and in the moister areas by the larvae, which have crept up blades of grass, coming into contact with the skin round the mouth during grazing. Infection in the former case can be considerably reduced by the liberal application of common salt round the drinking troughs as it has been found that the infective larvae are easily killed by a strong salt solution. Unfortunately no satisfactory drugs have been found which will expel the worms from the intestine, Carbon tetrachloride or Tetrachlorethylene, the commonly used drugs for the treatment of other hookworms, having so far given very disappointing results. However, better results may perhaps be obtained with these drugs when a more satisfactory method has been devised for introducing these drugs direct into the abomasum.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 3, Number 2, October, 1934.

On *Habronema murrayi* sp. n. from the Barn Owl—*Tyto alba.*

By R. J. ORTLEPP, M.A., Ph.D., Empire Marketing Board Research Officer, Onderstepoort, Transvaal.

THROUGH the kind offices of Mr. G. N. Murray, of this Institute, a barn owl was placed at the disposal of the writer for dissection. A careful examination of the digestive tract revealed the presence of 14 nematodes in the gizzard, some of which were lying free in its lumen, whereas others were embedded in its lining. All the other organs were free of helminths, but smears made from the heart blood, kidneys, and spleen were found by Mr. W. O. Neitz, of this Institute, to contain *Leucocytozoon*, *Haemoproteus* and *Trypanosomes*.

The worms are pinkish in colour, slender and attenuated towards their anterior end. The head is set off from the rest of the body by a slight constriction, and the cuticle shows very coarse annulations, which in optical section gives the sides of the body the appearance of being serrated; these annulations are about 0·0125 mm. from each other and in the oesophageal region are interrupted in both the lateral fields. There are no lateral alae, the area in which they normally occur being smooth and devoid of annulations. The cervical papillae are very small and peg-like and are lodged a little over half-way between the anterior extremity and nerve ring; in a 7·6 mm. long male they were 0·154 mm. and in a 11 mm. female 0·166 mm. from the anterior end. In these same two specimens the nerve ring was 0·23 mm. and 0·28 mm. from the front end respectively with the excretory pore some 0·05 mm. posterior to it in both cases.

The two lateral lips are very complicated and it was with difficulty that their true nature was made out. Each is tri-lobed, the middle lobe being the largest (Fig. 1). This lobe has a somewhat quadrangular shaft and from its antero-dorsal and antero-ventral

corners a curved horn-like process passes dorsalwards and ventralwards respectively on the inner side of the outer lobes. The outer lobes are somewhat rounded, their inner faces being concave and into this concavity the curved processes of the median lobe fit. The median lobes each carry on its inner face a tripartite tooth which is supported by a cuticular branch from the buccal capsule. In addition to these two trilobed lateral lips there is a small spike-like and keeled process on the dorsal and ventral side between the lateral lips, these may perhaps be regarded as of the nature of interlabia (Fig. 2). There are four submedian papillae, one near the base of each of the four outer lobes of the lips.

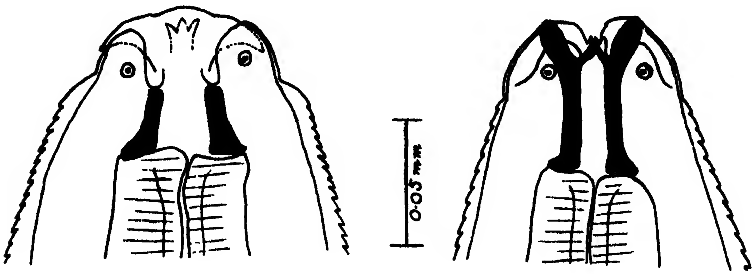
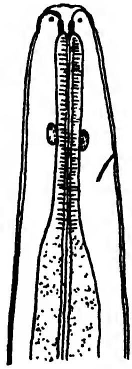


Fig. 1.—Anterior extremity, lateral view.
Fig. 2.—Anterior extremity, dorsal view.

There is a well-developed buccal capsule which appears deeper in a dorsal or ventral than in a lateral view (Figs. 1 and 2); this is due to the fact that it is continued as a process into each median lobe of the lateral lips; these processes are slightly club-shaped and extend to the anterior margin of the lips; about half-way up the lips each process gives off a fine branch on its inner side; these branches pass inwards and forwards and form the supports of the tripartite teeth. The buccal capsule in lateral view has about the same depth in both sexes (0·03 mm.), but its diameter is slightly greater in the female (0·028 mm.) than in the male (0·022 mm.).

The oesophagus is long and slender and consists of two distinct parts (Fig. 3); it occupies just under a third of the total body length in the female and about two-fifths of the body length in the male. In a 11 mm. female it was 3·5 mm. long and in a 7·5 mm. male it measured 2·7 mm. The muscular oesophagus forms about 1/9th of the total oesophagus in the female and about 1/7th in the male, and is slightly thinner in the latter, being 0·044 mm. in diameter as against 0·058 mm.; the glandular oesophagus increases slightly in thickness towards its posterior end; in the female the measurements are 0·11 mm. at its anterior end and 0·19 mm. at its posterior end, whereas in the male these measurements are 0·1 mm. and 0·16 mm. respectively.

Female.—The length varies from 10·75 mm. to 12 mm. and the body tapers towards both extremities; its thickest portion is in its middle where the diameter varies from 0·356 to 0·41 mm. The tail is short and bluntly-pointed and is from 0·186 mm. to 0·191 mm. long (Fig. 4).



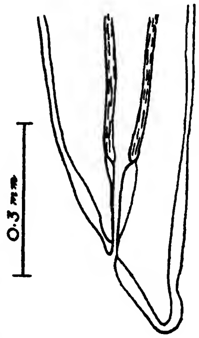


Fig. 3.—Anterior oesophageal region.
Fig. 4.—Caudal extremity of female.

The vulva opens just anterior to the middle of the body, being 5·4 mm. from the anterior end in a 11 mm. long female; its aperture is small and rounded and opens flush with the surface. The genitalia are of the type typical for the genus (Fig. 5); the vulva is small 0·038 mm. long by 0·022 mm. in diameter; this leads into the somewhat pyriform vestibule which is bent at its inner extremity; it is about 0·24 mm. long with a diameter in its middle of 0·07 mm. Its lumen is large and is lined by thick cuticle. The sphincter is elongate, 0·3 mm. long and has a uniform diameter of 0·032 mm.; its lumen also has a cuticular lining; the unpaired portion of the trompe is 0·22 mm. long and has an initial diameter of 0·045 mm., increasing to 0·077 mm. at the level of its two branches; these latter are the same length as the unpaired limb. The inner surface of this organ is lined by tall columnar cells, whose free ends meet each other in its lumen. At first the two uteri are parallel, but one soon bends back and passes forwards so that for their greater portion they are divergent. The uteri are filled with numerous eggs; these are oval, smooth, and thick-shelled and are embryonated prior to deposition; their average size is 0·047 mm. long by 0·028 mm. broad.

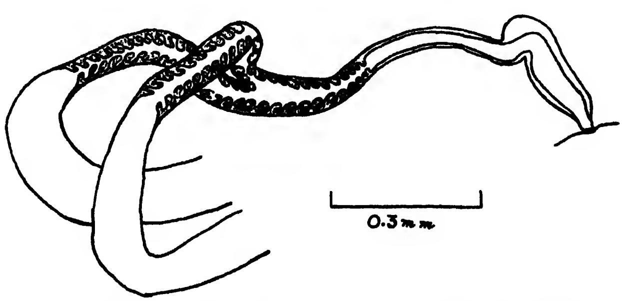


Fig. 5.—Terminal portion of female genitalia.

Male.—The males vary in length from 6·8 to 7·5 mm. and their maximum thickness is just anterior to the caudal expansions; from here the body tapers anteriorly, and whereas this also happens posteriorly, it is to a great extent masked by the large caudal expansions. The caudal extremity is generally hooked ventralwards, and in some it may even show a tendency to form a loose spiral. There are two large caudal expansions which are normally supported by four pairs of stalked preanal papillae, but this number is not always constant; e.g. in six males three had four papillae on either side, the fourth had five on the right and six on the left, the fifth had six on the right and four on the left, and the sixth had four on the right and six on the left (Fig. 6). The number and arrangement of

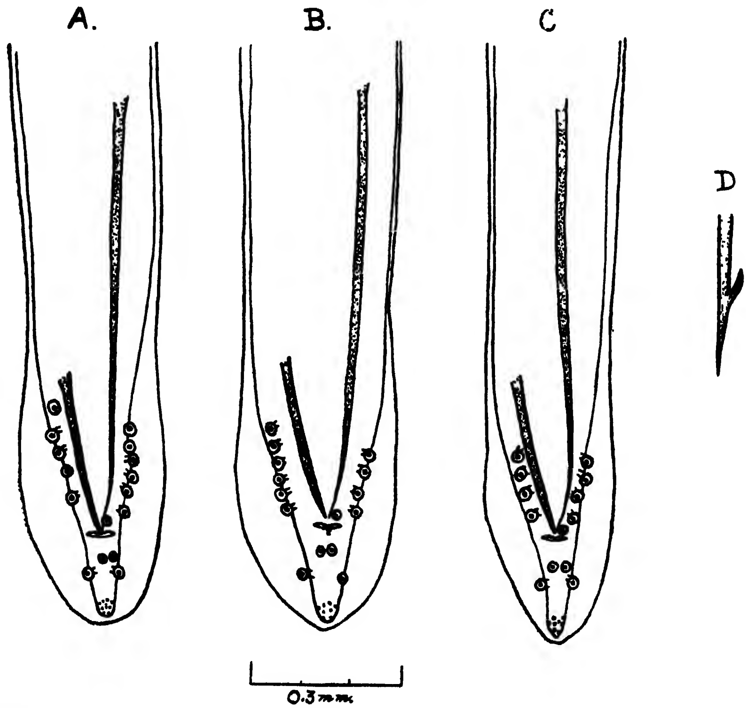


Fig. 6.—A, B, C, Ventral view of caudal extremities of three males. D, Tip of left spicule.

the post-caudal large papillae is constant; one pair is stalked and situated half-way down the tail, and another pair is sessile and approximated to each other and are found midway between the cloaca and the stalked papillae. In addition to these there is a group of about five pairs of very small papillae towards the tip of the tail. On the anterior lip of the cloaca there is a single large sessile papilla always situated towards its left corner. The whole ventral surface of the tail and its expensions is covered by elongated rugosities arranged longtitudinally; they are, however, entirely absent on a somewhat circular patch at the tip of the tail, and in the area anterior of this patch and posterior of the ventral postanal sessile papilla the rugosities are circular and not elongate.

The spicules are pointed, elongate and slender, that of the right being, however, slightly stouter than that of the left. The left spicule is from 0·884 mm. to 0·896 mm. long and 0·012 mm. thick, and about 0·03 mm. from its tip it carries a barb on one side. The right spicule is from 0·325 mm. to 0·332 mm. long with a maximum thickness of 0·019 mm. A somewhat trident-shaped gubernaculum is present.

Host: *Tyto alba.*

Location: Gizzard.

Locality: Onderstepoort, Transvaal.

Types in the Helminthological Collection, Onderstepoort.

Affinities.—The literature referring to 24 of the 25 known species of this genus reported from birds has been available; that not available was on *Habronem dipterum* Popowa, 1927. The absence of lateral alae separates the above described species from all the known species except from *Habronema ficheuri* Seurat, 1916, and possibly *H. casuaria* Maplestone, 1932; *H. euplocamu*, Maplestone, 1930; and *H. indica* Maplestone, 1929. No mention is made of alae in any of these species of Maplestone, so that their presence or absence is problematical. However, these three species all differ from the author's in that in them the vulva is situated towards the posterior extremity, whereas in the author's species its position is median.

The above described species shows close affinities to Seurat's species, the position of the vulva and nature of the female genitalia is similar in both, the annulations are interrupted along the lateral lines in both, and the tip of the left spicule is barbed in both, although in Seurat's species there are two hooks, in addition the lips also appear to be similar. However, it differs from Seurat's species in that the arrangement of the preanal caudal apillae is different, the left spicule is much shorter and carries only one hook.

[Addendum.—Since the writing of this article the author has collected this parasite from the Grass Owl, *Tyto capensis*, Onderstepoort.]

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Section IV.

Poisonous Plants.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 3, Number 2, October, 1934.

The Toxicology of Plants in South Africa.

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(Submitted in partial fulfilment of the requirements for the degree of Doctor of Veterinary Science, in the Faculty of Veterinary Science, University of Pretoria.)

GENERAL TOXICOLOGY OF PLANTS.

I. DEFINITION OF POISONOUS PLANTS.

II. CLASSIFICATION OF POISONS.

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B. *According to their chemical constitution.*

C. *According to similarity in pharmacological action.*

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C. *Absorption from the intramuscular tissues.*

D. *Absorption from the subcutaneous tissues.*

E. *Absorption from the respiratory tract.*

F. *Absorption from the alimentary canal.*

G. *Absorption from the skin.*

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I. *Absorption from the ear.*

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(*i*) Poisons affecting the liver.
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E. *Fighting the evil of overstocking.*

F. *Allowing animals access to water before they are allowed to graze on reaped lands where the edible herbage is dry and on which green and succulent poisonous plants occur.*

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SPECIAL TOXICOLOGY OF PLANTS.

I. The Problem of Geilsiekte.

II. Alopecia (Kaalsiekte) in Kids and Lambs caused by Plant Poisoning (*Chrysocoma tenuifolia* Berg).

THE TOXICOLOGY OF PLANTS IN SOUTH AFRICA.

I. DEFINITION OF A POISONOUS PLANT.

It is indeed a difficult task to define a poisonous plant in such terms as to comprise all plants which are considered detrimental to health. The most acceptable definition appears to be the following: A poisonous plant is a plant which, when consumed in such quantities as will be taken by animal or man over short or prolonged periods, exerts harmful effects on the system, or cause death by virtue of toxic substance(s) normally contained in that plant.

Many plants may at times constitute excellent feeds, whilst in certain circumstances they may prove deadly poisons. As examples mealie-stalks, sorghums, and a number of South African grasses may be mentioned. These plants are rightly considered valuable stock feeds, but in times of drought they may become deadly poisonous owing to the fact that they produce large amounts of prussic acid during the process of wilting.

Furthermore, many recognised valuable stock feeds are detrimental to health when fed in too large amounts over prolonged periods. As an example, cotton seed cakes and Lathyrus *sativus* L. may be quoted.

Another complicating factor in the definition of a poisonous plant is the fact that such quantities of a plant which are poisonous to one species of animal may not affect another species. Pigs are, for example, much more susceptible than sheep to poisoning with Syringa berries (*Melia azedarach* L.).

Again, some plants when eaten in small amounts may affect a certain organ or organs in such a way as not to cause immediate death or serious bodily harm, but may shorten life appreciably by decreasing the functions of such affected organs, and hence play an indirect rôle in the causation of death. Such plants are the species of *Senecio* concerned in stock poisoning and in bread poisoning in human beings. The seed heads and other parts of these plants become mixed with wheat threshed and milled in machines with deficient sieving and winnowing appliances and are then consumed with the bread. The active ingredients of these species of *Senecio* attack the liver and when taken in very small amounts over prolonged periods, cause cirrhosis of this organ. Apart from the ascites, which develops in human beings as a result of cirrhosis of the liver, the function of this organ, which is mainly a detoxicator, excretor and general protector of the system against all kinds of harmful substances, is markedly inhibited. Such affected subjects may, therefore, be much more susceptible to the effects of harmful substances which may find their way into the blood circulation via the liver, whether of exogenous or endogenous origin.

A part, or parts of a plant may be poisonous, whilst the remainder may be harmless and may even form part of the human or animal diet. This is the case in peach, apricot and prune kernels, which may frequently contain dangerous amounts of prussic acid, whilst the outer portion of the fruit is eaten with impunity. Similarly, the leaf-blades of rhubarb leaves are known to be poisonous, whilst the petioles are frequently used in the human diet.

The layman's idea of a poison is something that will cause death or disease when taken in small doses. The following examples will suffice to prove that plants may be the cause of serious bodily harm or even death in other ways than due to the poisonous constituents contained in them. In South Africa many plants are known to cause mechanical injury. The seeds of "steekgras" ("prick grass") (*Heteropogon contortus* R. & S., *Aristida congesta* R. & S., etc., etc.) may cause serious damage to the general health and condition of woolled sheep by irritating and piercing the skin, causing the penetration of pyogenic organisms which set up subcutaenous and in rare cases even intramuscular abscesses (Viljoen, 1918). From South West Africa cases have been reported where certain grass seeds have entered into the salivary ducts of cattle and have caused serious damage. Continuous ingestion of dry grasses rich in cellulose is prone to cause the formation of plant fibre balls in the stomach and intestines, especially in ruminants. This may lead to serious digestive disturbances and fatal obstruction of the gastro-intestinal canal. The seeds of burr-weed (*Xanthium* spp.) may cause partial or complete obstruction of the orifice of the sheath in oxen grazing on reaped lands overgrown with this weed. Such cases, if left unattended, may develop into severe inflammation of the sheath. The feeding of spiny prickly pear may lead to actinobacillosis and caseous lymphadenitis as was clearly proved by Thomas (1931). Some plants, for example lucerne, under certain conditions, cause death merely through the production of abnormal amounts of gas in the stomach, death apparently being due to asphyxia caused by high pressure on the diaphragm.

The constant feeding of plants deficient in minerals (stiffsickness, stywesiekte), proteins, lipoids (Jaffe, 1929), vitamins, etc., also lead to fatal results.

II. CLASSIFICATION OF POISONS.

Poisons may be classified as follows:—

(A) According to their origin: vegetable, mineral, animal, etc. This classification is obviously too vague to be of any value.

(B) According to their chemical constitution: alkaloids, glucosides, toxalbumins, picrotoxins, etc. Also this classification is too indefinite.

(C) According to similarity in pharmacological action, e.g.:—

(a) *Blood poisons:* acting on the blood.

(i) Poisons affecting cellular elements. Those acting on the red cells in particular and to a lesser extent on the white cells; for example, carbon monoxide, prussic acid, arsine, chlorates.

(ii) Poisons affecting the plasma. Those acting on the plasma as well as on the corpuscles or on the plasma only; for example, silver salts.

(*b*) *Neurotic poisons:* those acting on the nervous system.

(i) *Paralysomotor poisons.* Those affecting particularly the motor nerves, for example, curare and aconite.

(ii) *Spinal poisons;* for example, strychnine and yohimbin.

(iii) *cerebro-spinal poisons;* for example, morphine, chloroform and chloralhydrate.

(*c*) *Neuromuscular poisons:* those acting on the muscular system through the nerves controlling the muscles in question; for example, digitalis.

(*d*) *Muscular poisons:* those acting on the muscles without the intervention of nerves; for example, veratrin, lead and mercury.

(*e*) *Irritant or corrosive poisons;* for example, acids and alkalis in high concentrations, and corrosive sublimate.

To group C poisons, which attack certain organs only, may be added. The following might be cited as examples, *Senecio spp.*, which attack the liver, *Crotalaria dura* Wood & Evans, and *Crotalaria globifera* E. Mey, which attack the lungs in horses, and *Crotalaria burkeana* Benth which affect the hoofs in cattle.

Of the above schemes of classification, that given under group III appears most acceptable, although it is by no means satisfactory. Many poisons have complex actions and cannot be included under any one group to which a particular action is ascribed. It is for this reason that such great difficulty is experienced in the classification of poisonous plants, as many of them have most complex actions due to the presence of more than one substance, which is harmful to the system. In addition, the effects of one and the same plant may vary considerably according to the size of the dose administered and according to the method of administration.

Popularly poisonous plants are referred to as poisons to the brain, spinal cord, heart, liver, kidney, lungs and metabolism.

III. ACCESS OF POISONS TO THE ANIMAL BODY.

Poisons may find their way into the blood stream in the following ways:—

A. *Direct Introduction into Veins or Arteries.*

In this case symptoms of poisoning set in immediately or a short time after administration and death may occur instantaneously, as there is little or no time for elimination and inactivation of the poison. If a leathal dose of strychnine be injected intravenously the patient will in most cases succumb before the injection is completed (Steyn 1931d).

B. *Absorption from the Intraperitoneal and Intrathoracic Cavities.*

Serious membranes are very active absorbers, and fluids or substances in solution introduced intraperitoneally or intrathoracically are at once exposed to a large and active absorbing surface. Poisonous substances introduced into the body by this method will therefore exert their toxic effects very soon after administration, for the same reasons as mentioned above.

C. *Absorption from the Intramuscular Tissues.*

The rate of absorption of substances administered by this method is almost as quickly as in the case of intravenous injections. In the case of wounds brought about by poisoned arrows, blood vessels are ruptured and the poison is to a certain extent introduced directly into the blood circulation.

D. *Absorption from the Subcutaneous Tissues.*

Here the rate of absorption is slower than when the poison is administered intravenously or intramuscularly, as, unless the subcutaneous tissues are damaged over a large area with the consequent severing of bloodvessels, absorption into the blood circulation has to take place by way of osmosis. Irritant substances (oil of turpentine) introduced subcutaneously cause local inflammation with consequent necrosis in cases of severe irritants.

E. *Absorption from the Respiratory Tract.*

Absorption of gases and vapours by the lungs is practically instantaneous, the surface exposed to the action of inhaled gases being enormous, hence facilitating absorption. Also liquids are rapidly absorbed by the lungs, and even inhaled solids, when these are in a state of fine division, are allowed to pass into the lung tissues (silicosis). It is, for example, possible to cause death in guinea-pigs from ricinus (castor bean) poisoning by allowing these animals to inhale the dust of the beans (Ratnet and Gruehl, 1927-1928).

F. *Absorption from the Alimentary Canal.*

The amount of poison absorbed from the intact buccal cavity, pharynx and oesophagus is in most cases negligible, as it is rarely retained here for any length of time. Most poisons when retained for any length of time in the buccal cavity will be absorbed to a certain extent (Blume and Buchholz, 1932). Discussing absorption by the mouth Witthaus (1911, p. 83) writes: "Poisons of great activity, however, enter the circulation from the mouth or lips or the nasal mucous membrane with such rapidity that their contact with those surfaces may cause death before an act of swallowing is attempted, or even possible. Thus Preyer found that rabbits fell unconscious in twenty-three seconds after the application of hydrocyanic to the tongue, and in twelve seconds after its application to the nostrils. Nicotin also, when applied to the mouth in doses of two to four drops, may cause poisoning either immediately or within half a minute. In cases of tobacco poisoning from excessive smoking, in which the smoke is not inhaled, the absorption is by the buccal mucous membrane, except in so far as it may occur by swallowed

saliva. Arsenic is also absorbed from the mouth under exceptional circumstances, as in those instances in which arsenical symptoms were traced to smoking tobacco or cigars impregnated with arsenic. Gorochofzeff has shown that dogs after ligation of the oesophagus are killed by strychnine more rapidly by the mouth than by the stomach." Irritant poisons, which cause partial or complete destruction of the mucous membrane, will be absorbed in the same way as from wounds.

Absorption of poisons from the fore-stomachs of ruminants is negligible, except in case of gases and volatile substances, which will diffuse through the mucous membrane and in this way reach the blood circulation. In experiments conducted at Onderstepoort by the author it was found that sheep drenched by stomach tube with highly lethal amounts of *Dimorphotheca spectabilis* Schltr. (bietou) were already in a state of collapse by the time the stomach tube was withdrawn, thus indicating that the prussic acid contained in this plant passed through the ruminal wall as there was no time for the material drenched to find its way into the abomasum and small intestine. Volatile substances, like ether and alcohol, also to a large extent pass through the ruminal wall. Absorption by the stomach (abomasum in ruminants) is as a rule slow, depending on the diffusibility and solubility of the poison, and what has been said with regard to absorption by the fore-stomachs, also applies here. The following passage quoted from Witthaus (1911, p. 84) is of interest: "There are differences in the rates of absorption of certain substances from the stomach in the different kinds of animal. Thus strychnine is absorbed more slowly in cows and much more slowly in horses than in dogs, pigs or cats. Otto found that strychnine is absorbed from the ligated stomach in cats and dogs, but not in rabbits or guinea-pigs, while the reverse is the case with potassium iodide and sodium salicylate ". Poisons insoluble in watery fluids but soluble in oils are not absorbed by the stomach. Such substances, for example phosphorus, are absorbed from the intestine and reach the blood circulation via the thoracic duct, provided the mucous membrane is not damaged, in which case we must accept that the phosphorus will be absorbed from the stomach.

The small intestine, being the most active organ of absorption, also very readily absorbs poisons. It is well known that foods, drugs and poisons in solution are rapidly absorbed by the rectum, some drugs and poisons (atropine, morphine) being even more rapidly absorbed than when given by the mouth.

G. *Absorption from the Skin.*

The intact skin also is an active absorber of most poisons soluble in lipoids and in water. The fact that the application to the skin of ointments and dipping fluids containing drugs, in too strong concentrations, or over prolonged periods, have been responsible for serious and even fatal poisoning, is too well known to be mentioned. It is also known that drugs incorporated in lanolin are more readily absorbed than when other fats are used as vehicles. The vapours of certain poisons (mercury) and even finely divided powders, especially when friction is applied, are also absorbed by the intact skin. Such

substances find their way into the blood circulation probably via the sebaceous glands. It stands to reason that absorption from the skin will be facilitated when friction is applied.

Absorption from the damaged skin will take place in a manner similar to that found in the case of wounds. Irritants, which will not penetrate the intact skin in normal circumstances, will do so readily after having destroyed the superficial layers of this organ.

H. *Absorption from the Genito-urinary Tract.*

The genito-urinary tract also is a fairly active absorber of poisons. In this connection many accidents have happened in the treatment of affections of the urinary bladder and uterus. In the post-parturient state the uterus which is a much more active absorber than the bladder, absorbs drugs much more readily than at other times. In olden times the introduction of arsenic into the vagina was not an uncommon method used in criminal poisoning. According to Witthaus (1911) atropine, but not strychnine, is absorbed from the urinary bladder. Here also absorption from the intact and damaged mucous membrane must be distinguished.

I. *Absorption from the Ear.*

Non-irritant substances (atropine) in solution may be absorbed from the auditory canal. In criminal poisoning mineral acids and solutions containing arsenic were poured into the ear of sleeping victims. In these cases absorption was very rapid as the mucous membrane was corroded. Accidents have frequently occurred in the treatment of ear parasites in animals, especially with arsenical solutions.

J. *Absorption from the Eye.*

It has been established that atropine is absorbed from the intact palpebral and corneal conjunctiva, absorption is, however, slower than from mucous surfaces.

K. *Absorption from the Nasal Mucous Membrane.*

The intact mucous membrane of the nose may also absorb drugs and poisons although absorption will in most cases be very slow and never, or very rarely, lead to poisoning unless poisonous substances are applied over prolonged periods or to the damaged mucosa.

Mechanism of Absorption.

The principle underlying absorption is osmosis. Crystalloids (salts, sugar) readily diffusing through the cell membranes, whilst colloids as such (mucilages, proteins) pass through thee membranes very slow or not at all. Most gases (prussic acid) diffuse extremely rapidly through the cell membrane.

Poisons absorbed from surfaces other than that of the gastro-intestinal tract find their way directly into the main circulation. whilst those absorbed from the stomach and intestines (with the exception of fat soluble substances, which enter the blood circulation through the thoracic duct) enter the liver by way of the portal vein.

Many plants contain active ingredients, which have an irritating effect on the gastro-intestinal mucosa. In these cases the mechanism of absorption is rendered abnormal owing to damage done to the mucous membrane, and the poisoning is further complicated by the fact that harmful substances, which are present in the gastro-intestinal tract in normal circumstances, will find their way into the blood circulation. It is for this reason that a certain proportion of the phosphorus dissolved in oil in such concentrations as would damage the gastro-intestinal mucosa, will find its way into the liver through the gastric and mesenteric veins.

IV. THE FATE OF PLANT POISONS IN THE BODY.

The action of body fluids on the toxic constituents of plants from the time they enter the mouth up to the point of absorption by the gastro-intestinal mucosa will be discussed under "Factors concerned in the Determination of the Toxicity of Plants". Very little is known about the changes induced in the toxic constituents of plants and of their fate after absorption into the blood circulation. In this respect more definite information is available in connection with the mineral poisons.

In discussing the fate of poisons in the body we have to consider that there are two ways in which poisons absorbed by the intact gastro-intestinal mucosa may find their way into the general circulation. Most of these poisons find their way directly into the liver through the gastric, mesenteric and portal veins, whilst those, which are soluble in fats only, enter the general circulation via the thoracic duct. These poisons passing through the liver undergo, as a rule, certain changes. The liver, as the main protector of the body against the effects of poisons, arrests, retains, modifies and excretes poisons, and the higher its glycogen content the greater its detoxicating capacity. The toxic constituents of plants may be deposited in the liver by the formation of insoluble or slightly soluble compounds (for example, combinations of alkaloids and biliary acids). In addition, processes of neutralisation, oxidation, reduction, synthesis and decomposition may play an important rôle in the detoxication of poisonous substances in the liver and other organs, and also in the tissues and fluids of the body. Some poisons are deposited in one or other of the organs, for example, strychnine in the central nervous system.

Witthaus (1911, p. 104), referring to experiments conducted by Czylharz and Donath said that they concluded, "that the subcutaneous cellular tissue, the muscles, or the body or lymph has the power to neutralise strychnine during life". Dold (1914) found that fresh serum detoxicates all extracts of organs and that this detoxicating effect of serum is lost by heating it to 60° C. or by filtering it and Beutner (1926) established the fact that bovine and rabbit serum binds pilocarpine in such a manner that it can again be recovered by precipitating the serum. It is a well established fact that the body produces specific antitoxins to toxalbumins (ricin, modeccin, abrin, etc.).

Our knowledge of the distribution in the body and elimination from the body of plant poisons, especially those of South African origin, is very limited. A poison may be eliminated by certain or all organs depending on the nature of the poison. The rate of elimination, which is of so much importance in the determination of the toxicity of a substance, depends on the nature of the poison, on the organ or organs concerned in the elimination, and on the state in which these organs are. Gaseous and volatile poisons (prussic acid, ether, etc.) are mainly excreted by the lungs and skin, whilst in the elimination of other poisons the kidneys, liver, mucosa of the alimentary canal (salivary glands, gastro-intestinal glands), skin (talc and sweat glands), bronchial glands and lactating glands are concerned. Many poisons are eliminated as such (prussic acid) whilst others (carbolic preparations) are changed in the body and then excreted. Oxalic acid forms insoluble calcium oxalate in the blood, and the biliary acids from salts with alkaloids, which, being soluble in bile, are passed out with the bile and may be reabsorbed or passed out with the faeces. Again, some poisons (gases) are eliminated at a very quick rate, whilst others may take months to leave the body. This fact brings us to the cumulative effects of poisons. It is difficult to determine exactly which poisons have a cumulative effect and which not, as this effect is determined by the rate of absorption and elimination of a poison. Substances, which are absorbed quickly and eliminated slowly, will naturally have a tendency to accumulate in the body. The size of the dose and the intervals of dosage must therefore be considered when ascertaining whether a poison will have a cumulative effect or not. Poisons, which are fixed for a time by certain organs will have a tendency to exert cumulative effects. It is well known that digitalis has cumulative effects and that care must be exercised in the treatment of chronic heart diseases with this drug. It should also be mentioned here that all poisons are likely to have cumulative effects, when the organs of excretion especially the liver and kidneys are diseased, thus allowing of very slow excretion. According to Seni (1929) prussic acid is to a certain extent destroyed by blood.

Weese (1930) states that Cloetta and his collaborators have shown that striated muscle has a highly specific capacity for the fixation of digitoxin. This glucoside is bound irreversibly and is slowly decomposed into aglycon and sugar by the action of ferments. Weese holds that the cumulative action of digitoxin is attributable to the fact that it is fixed in the heart muscle, while that portion of the digitoxin that is bound extra-cardiacally is decomposed or excreted.

V. MODES OF ACTION OF PLANT POISONS.

A. Local Action.

We must distinguish between local and remote action of poisons. The local action is that which is exerted at the point of application, whilst the remote action of a poison occurs only after absorption into the blood circulation. Many plants taken per os have an irritating effect on the gastro-intestinal mucosa. This effect may be due to the toxic ingredient or ingredients (digitalis glucosides), or to one or other substances (acids) which such plants may contain in addition

to the active principle, which is mainly responsible for death. *Psilocaulon absimile* N.E. Br. contains a large amount of acid oxalates and also a narcotic alkaloid (Rimington and Steyn, 1933). Some plants (*Moraea* spp. *Homeria* spp.) cause severe irritation and even destruction of the mucosa of the gastro-intestinal tract, with the result that the mechanism of absorption is rendered abnormal. Noxious substances, which are present in the digestive tract in normal circumstances and which are not absorbed by the intact mucosa, may find their way into the blood circulation and will exert their harmful effects on the system, thus aggravating the condition of the already poisoned victim. It is, therefore, possible that a plant may cause death by virtue of its irritating properties only. Death in such cases is probably not due to some or other deadly poison contained in the plant, but to poisonous substances normally present in the digestive tract and which are not allowed to pass through the intact mucous membrane. In addition, the disturbed digestion may be the cause of the production of further harmful substances in the gastro-intestinal tract. Before concluding the remarks on local action, it must be mentioned that many plants (*Euphorbia* spp.) contain irritant juices, which, even when applied to the skin, cause severe inflammation.

B. Remote Action.

It is hardly necessary to state that it is an impossibility to cover fully the vast ground of the remote actions of all plant poisons. Therefore only a few of the most outstanding examples will be referred to under this heading.

After absorption into the blood stream plant poisons may exert their effects on the body in many ways. The rôle played by the liver in the inactivation and elimination of poisons has already been referred to under " The Fate of Plant Poisons in the Body ". Some poisons have a special affinity for and may be fixed by certain organs; hence we speak of liver, kidney, heart and nerve poisons. Poisons fixed by certain organs are inclined to have cumulative effects (digitoxin). Most poisons exert their effects on all the organs to a certain extent. As an example of the manner in which poisons may affect the system, the following instances may be quoted:—

(a) *Poisons Affecting the Blood.*

(1) *Red blood corpuscles.*—Poisons may change the blood by causing haemolysis (saponins), methaemoglobinaemia (nitrites, chlorates) or by attaching themselves to the red blood corpuscles and replacing the oxygen (carbon monoxide).

In these cases death is due to asphyxia as the oxygen-carrying capacity of the blood is destroyed.

Annau and Hergloz (1928) found that chronic strychnine poisoning in rabbits was accompanied by a diminution of 35 per cent. in the total red count, whilst in splenectomized rabbits suffering from chronic strychnine poisoning there was an increase of about 35 per cent. in the total red count. The latter increase is considered by them to be due to stimulation of the reticulo-endothelial apparatus.

(2) *Leucocytes and lymphocytes.*—The movements of these blood corpuscles are paralysed by some poisons (quinine), while other poisons (strychnine) again cause a hypoleucocytosis.

(3) *Serum.*—The serum is coagulated by some poisons (ricin, abrin), the result being the formation of emboli and thrombosis.

(*b*) *Poisons Affecting the Heart.*

Of the indigenous South African poisonous plants, which are heart poisons, *Dichapetalum cymosum* (Hook) Engl. (gifblaar) and *Pachystigma pygmaea* Schltr. Robyns (gousiekte bossie) may be mentioned, the latter plant causing a chronic indurative myocarditis.

Heart poisons may directly affect the heart muscle, or may affect it through the vagus and accelerator nerves, or they may affect both. Digitalis affects both the heart muscle and the vagus nerve.

(*c*) *Poisons Affecting the Blood Vessels.*

Blood vessels may be dilated or constricted by poisons. Ephedrine and histamine cause contraction of some vessels, and dilation of others, whilst digitalis causes constriction. These effects are caused by the action of the poisons either on the centres of vasodilation and vasoconstriction, or on the nerve-endings, or on the muscles in the walls of the blood vessels.

(*d*) *Poisons Affecting the Respiratory System.*

These include poisons either affecting the centre of respiration, or the vagus-endings in the lungs, or the bronchial glands or the muscles, larynx muscles, muscles of respiration (intercostal muscles and diaphragm), on the blood vessels in the lungs, etc., etc. Slowing and weakening of the respiration may be brought about in the following ways: (1) Paralysis of the centre of respiration (prussic acid); (2) paralysis of the muscles of respiration (curare); (3) paralysis of the vagus-endings in the lung (atropine), or (4) stimulation of the inhibiting centre of respiration. Stimulation of respiration can be achieved in the reverse way. Oedema of the lungs may be effected by one or more of the following conditions: (i) increased secretion of the bronchial glands (pilocarpine, arecoline); (ii) hyperaemia of the lungs; and (iii) increased permeability of the vessels in the lung. As an example of plants exerting a local effect on the lungs or the liver or both *Crotalaria dura* Wood and Evan's and *Crotalaria globifera* E. Mey. may be mentioned.

(*e*) *Poisons Affecting the Nervous System.*

Different poisons may affect different parts of the central nervous system and their effects may be of a stimulatory or inhibitory (narcotic) nature. The brain is stimulated by atropine producing hallucinations, excitement, and epileptiform convulsions, whilst morphine, after a preliminary stage of excitement, which varies in different individuals, cause depression, sleep and coma. The centres (heat regulation, respiration) situated in the medulla oblongata are affected by many poisons.

Strychnine is known to exert its main effects on the spinal cord.

The following plants indigenous to South Africa exert their effects mainly on the central nervous system: *Cynanchum africanum* R. Br., *Cynanchum capense* Thunb., *Cynanchum obtusifolium* L.f., *Matricaria nigellaefolia* DC., and various *Cotyledon* spp.

A typical example of poison affecting the peripheral nerves is curare, a South American arrow poison.

(*f*) *Poisons Affecting the Gastro-intestinal Canal.*

Poisons may affect the digestive tract in various ways. Many substances cause severe irritation of the mucosa and even perforation of the gastro-intestinal wall, while others decrease or increase the gastro-intestinal movements and secretions either through acting on the wall directly or through the nerves, or nerve endings. Stimulation of the vagus causes increased movements of the stomach, whilst splanchnic stimulation retards intestinal peristalsis. Many of the South African poisonous plants are gastro-intestinal irritants in addition to causing other symptoms of poisoning.

(*g*) *Poisons Affecting the Kidney.*

Harmful substances may stimulate, irritate or even cause necrosis of the epithelium in the uriniferous tubules. If the epithelium is stimulated or irritated diuresis occurs, whilst in the case of glomerulo-nephritis oliguria and anuria may supervene. Irritant substances excreted by the kidneys may cause irritation of the mucosa of the ureters, urinary bladder and urethra. Haemolytic poisons, in themselves harmless to the kidneys, cause irritation of the epithelium through the excreted haemoglobin. Chronic irritation of the kidneys will ultimately lead to fibrosis of their tissues.

(*h*) *Poisons Affecting the Uterus.*

Poisons may exert their effects (inhibition or stimulation) on the uterus in the following ways: (1) through the uterus-centre situated in the lumbar region (strychnine); (2) through the uterus ganglia; and (3) by affecting the musculature itself. The uterus is much more susceptible to drug-action in the pregnant than in the non-pregnant state. Many plants containing severe gastro-intestinal irritants may cause abortion, probably in an indirect manner.

(*i*) *Poisons Affecting the Liver.*

All poisons passing through or retained by the liver probably exert, to a certain degree at least, harmful effects on this organ, resulting in regressive changes (e.g. atrophy, degeneration necrosis) or progressive changes (e.g. inflammation, cirrbosis) or both. An outstanding example of plants which mainly affect the liver is certain species of *Senecio*. With regard to the functional activity of the liver acted on by certain poisons, Whipple and Speed (1915) state: " It has been established that specific liver poisons (e.g. chloroform, phosphorus) causing histological changes in the liver cells, decrease the liver excretion of phenoltetrachlornaphthalein. Also vascular disturbances (Eck fistula, passive congestion) with or without histological evidence may cause a fall in the output of naphthalein through the liver. Sufficient evidence has been brought forward to show that the phenoltetrachlornaphthalein excretion is a valuable

index in respect of the functional activity of the liver. Ether anaesthesia for a period of two hours usually causes a depression in the naphthalein curve during the twenty-four hours following the anaesthesia.

Paraldehyde in doses sufficient to give anaesthesia and stupor for a few hours will give a definite fall in naphthalein excretion.

Chloral and urethane usually cause a decrease in naphthalein output when given in considerable amounts.

Alcohol causes a drop in the naphthalein curve when given in large doses sufficient to cause stupor for a few weeks. The drop in phenoltetrachlornaphthalein excretion is demonstrated in the twenty-four hours following administration of the drug. A drop in the naphthalein curve to two-thirds or one-half of normal indicates a definite liver injury and temporary impairment of function."

(j) *Poisons Affecting the Salivary Glands.*

Poisons may cause increased salivation by (1) stimulation of the salivary centre (pilocarpine); (2) stimulation of the peripheral endings of the salivary nerves (pilocarpine); (3) stimulation of the peripheral taste nerves; and (4) stimulation of the gland-cells themselves. Decrease in salivation will be caused by reverse processes, atropine, for example, inhibiting salivation by paralysing the nerves concerned.

(k) *Poisons Affecting Sweat Glands.*

The actions of poisons on the sweat glands are analogous to those described under " Poisons affecting the salivary glands ".

(l) *Poisons Affecting the Eye.*

Poisons may affect the muscles controlling the movements of the eye either directly or through their nerves. Myosis may be caused by (1) paralysis of the mydriatic centre in the brain (morphine in the dog); (2) peripheral stimulation of the oculomotor nerve; and (3) stimulation of the sphincter muscle in the iris (eserine). Mydriasis, on the other hand, may be effected by (1) paralysis of the oculomotor nerve (atropine); (2) stimulation of the mydriatic centre in the brain (morphine in the cat); and (3) peripheral stimulation of the mydriatic nerves.

(m) *Poisons Disturbing Processes of Metabolism.*

The means at our disposal of studying this most involved problem are very imperfect, and at present it can only be investigated to a certain extent by chemical examination of urine, faeces, and exhaled air. Many poisons cause a decrease in the oxidation processes in the body by (1) decreasing the oxygen carrying capacity of the blood (carbon monoxide, chlorates); (2) by preventing the red blood corpuscles from disposing of the oxygen they have conveyed to the tissues (prussic acid), or (3) inhibiting the intracellular processes of metabolism through partial or complete paralysis of the cells as in the case of protoplasm poisons (quinine). The processes of metabolism may, on the other hand, be increased by certain poisons acting on the centre of heat regulation.

Kahn and Goodridge (1926, p. 373) write: "Loevy, in 1907, demonstrated that hydrocyanic acid not only increased the protein catabolism, but also influenced the metabolism qualitatively. Wallace and Richards studied the effect of potassium cyanide upon metabolism and they observed that the total sulphur output was increased on the day of poisoning but, unlike the total nitrogen, it fell on the following day. The neutral sulphur fraction was increased, whereas the sulphate sulphur was diminished, showing that the oxidative processes in the body were lessened".

In strychnine poisoning there is an increase in the calcium content of the blood serum, which is due most probably to calcium being forced out of the muscles during attacks of spasms (Beznák, 1931).

Cutler (1932) found that within a few hours after the administration of carbon tetrachloride the guanidine content of the blood is increased and suggests that carbon tetrachloride poisoning is largely due to this guanidinaemia. There is a severe disturbance of carbohydrate metabolism, the blood sugar content, after a short preliminary rise, falling to a very low level. The quanidine present in the blood interferes with the oxidation processes in the tissues with a consequent rise of the lactic acid content of the blood and urine owing to the inability of the tissues to reconvert lactic acid into its precursors. The result is that the carbohydrate stores of the body are rapidly depleted and the hypoglycaemia appears to be the immediate cause of death. At the time of death the glycogen content of the liver is very low.

In animals poisoned with thallium the lipoid metabolism is disturbed, as is evidenced by the fact that there is a marked decrease in the lipoid content, or, even a complete disappearance of lipoids from the adrenal cortex, the skin and nervous system, (Buschke and Peiser, 1932).

Oxalic acid absorbed into the blood combines with the calcium present in the blood liberating potassium and sodium with the result that the ratio $\frac{Na+K}{Ca+Mg}$ is upset and an alkalosis produced.

(n) *Poisons Affecting other Organs.*

Stiffsickness ("stywesiekte") caused by the ingestion of *Crotalaria burkeana* Benth may be mentioned here, as it affects the hoofs of cattle. In connection with plants which affect the skin *Chrysocoma tenuifolia* Berg, the cause of alopecia in kids and lambs may be referred to here. It is not known whether loss of hair is due to the poison acting on the skin directly or whether it is caused indirectly through endocrine sympathetic disturbances. From preliminary investigations made it would, however, appear that the loss of hair is due to the latter disturbance, as is thought by some authorities to be the case in alopecia caused by thallium poisoning (Buschke and Peiser, 1932). Mackay (1931) produced a pronounced increase in the weight of the adrenal glands of white rats by repeatedly dosing them with morphine sulphate.

The fact that quite a number of hypothesis have been put forward to explain the action of poisons on the cells and tissue elements, is ample proof that very little is known about it. There are hypotheses which postulate that the reactions of cells to poisons are due to chemical changes induced in the cells affected, or changes in the interrelationship of the cell constituents, and that this modification is associated with a change in the electrical charge, which in turn causes the cell to react in certain ways. The similarity in the action of chemically closely related poisons and the fact that the action of such related poisons can, to a certain extent, be deducted from their structural formulae, have influenced some investigators to advance the hypothesis that the actions of poisons depend directly upon their chemical structure.

The discussions of Henderson (1930) and Henderson and Lucas (1932) in connection with the theories of narcosis are most interesting. They refer to the theory of Bibra and Harless, to the theories of precipitation, dehydration, water solubility, asphycia, absorption, permeability, and also to those postulated by Traub, Meyer-Overton (lipoid theory), Beutner, and Claude Bernard. The last-named authority advanced the theory that narcosis is due to the fact that narcotics cause a state of reversible semi-coagulation of the substance of the nerve cells.

There seems little doubt that the actions of the different poisons are exerted in different ways and that these actions depend on a large number of factors about which we know very little at the present time.

Acute and Chronic Poisoning.

Before concluding this chapter acute and chronic poisoning must be referred to. The quantity of a poison ingested and the time within which it is taken are the factors which determine the symptom complex in the affected subject. Highly lethal quantities of a poison taken in one dose may cause sudden death, whilst repeated small doses may cause symptoms of poisoning over prolonged periods. With regard to poisonous plant the course of poisoning (peracute, acute, subacute and chronic) is determined by the nature and amount of poison present in the plant and the time in which any quantity is taken.

Again, some plants may cause death very soon after ingestion (plants containing prussic acid), whilst others exert their actions only when a certain period has elapsed after ingestion (*Pachystigma pygmaea* Schltr. Robyns; *Crotalaria dura* Wood & Evans). This period that elapses between the discontinuation of feeding on a plant and the appearance of symptoms has in the past frequently been referred to as " incubation period ". The term " incubation period " should, however, be applied only to those cases where organisms (bacteria, protozoa) finding their way into the system, are capable of producing poisons (toxins) in the body. In plant poisoning, accumulation of poison in the body is only possible when the plant is eaten repeatedly within certain time limits. The term " period of latency " is suggested for the period of time which elapses between

the discontinuation of feeding with the plant and the appearance of symptoms, as the period of latency is quite different from that termed "incubation period" in infectious diseases.

It is indeed a difficult task to determine the toxic and lethal doses of plants which exert their effects after a certain period of latency only. In order to elucidate this point, it is necessary to consider why certain plants behave in this way. There are two possibilities for this behaviour, namely, (*a*) in the case of a certain number of these slow acting poisons it may be necessary for them to accumulate in the body of the animal before clinical symptoms appear; and (*b*) each small quantity of the poison taken in repeatedly may cause progressive processes of disease (degeneration, inflammation, etc.) in the organs. Do these processes continue after every trace of the poison had been eliminated from the body, or does the undamaged portion of this organ become affected when it is subjected to sudden increased function (resulting from sudden changes in diet, excessive exercise, etc.)? Such an affected animal may then die with symptoms of staggers resulting from further elimination of liver tissue.

Experience gained with plants like *Pachystigma pygmaea* (Schlt.) Robyns, *Crotalaria dura* Wood and Evans, *Crotalaria globifera* E. Mey., and various species of *Senecio*, support the latter view. It is well known that animals may still die from poisoning many months after having ingested these plants. Well marked lesions may be present in one or more organs without any clinical symptoms being discernible. The matter is further complicated by the fact that in *Crotalaria* poisoning in the majority of the horses there is a chronic pneumonia, whereas in Seneciosis the liver is severely damaged. It is obvious that such affected animals are much less resistant to diseases and poisons than normal animals.

These few remarks will suffice to point out the difficulty of determining the toxic and lethal doses of plants with a long period of latency.

VI. THE TOXIC PRINCIPLES OF PLANTS AND THEIR PHYSIOLOGICAL SIGNIFICANCE.

The toxicity of plants may be due to the presence of one or more of the following substances: alkaloids, glucosides, resins, picrotoxins, toxalbumins, volatile oils, alcohols (tremetol) and organic acids (oxalic acid and its acid salts). Although a number of investigators have interested themselves in this aspect of poisonous plants, our knowledge of the active principles of plants indigenous to South Africa is very limited indeed.

As a rule closely related plants have the same, or, chemically and pharmacologically closely related, active ingredients (solanin is present in a number of *Solanum* spp.), whilst in other cases such plants contain toxic principles different in their chemical nature and more of action (*Strychnos Nux-Vomica* L.) contains strychnine, which affects the spinal cord, whilst the amorphous alkaloid of *Strychnos Henningsii* Gilg. exerts its effects on the medullary centres

(Rindl, 1931). Again, the same, or similar active principles, may be found in plants belonging to different families as in the case of saponins and prussic acid.

Much has been written and said about the physiological significance of the toxic principles of plants and a number of theories have been advanced, but nothing definite is as yet known. Three main ideas are held in this connection, namely, that toxic substances contained in plants are (*a*) products of catabolism (excretory products), (*b*) produced in order to protect plants against being eaten by man and animal, (*c*) stages in the processes of anabolism in the plant. Various facts may be mentioned to prove and disprove each of these hypothesis and it seems quite feasible that one or more of the abovementioned points may be responsible for the production of poisons in plants. The least acceptable suggestion is that a plant produces a poison in order to protect itself against being eaten by man and animal. Why should some plants and not others protect themselves? If this were the case it would appear likely that the plants most eaten by stock would be the most likely ones to produce poison in order to prevent them from being eradicated. If plants produced poisons in order to protect themselves they would most likely have concentrated these poisons in the parts growing above ground. This is not the case with many poisonous plants, as their roots or bulbs contain the largest proportion of the poison. Again, why should plants protect themselves only under certain climatic conditions, whilst at other times they are valuable stock-feeds? This is the case with *Tribulus terrestris* L. (duwweltjie), which in certain areas and under certain climatic conditions causes "geel-dikkop" ("yellow thick head"), whilst at other times it saves thousands of animals from starvation. This also is the case with a number of our most valuable veld grasses (see *Gramineae*), which when wilted and stunted often produce deadly amounts of prussic acid. Furthermore, it is inconceivable why plants growing in certain areas should protect themselves to a greater degree than those of the same species growing elsewhere. It is a well-known fact that members of the same species of plant growing in different areas may vary considerably in their toxicity, and striking variations in toxicity have been found even in members of the same species of plant growing beside each other.

The fact that many plants concentrate their toxic principles in the bark is held to be a proof of the hypothesis that these principles are excretory products of such plants. Such poisons are "eliminated" by the plant casting the bark.

The facts that many plants are more poisonous in the earlier than in the later stages of development (the development of prussic acid in *Sorghum vulgare* Pres. and *Linum usitatissimum* L.), and that poisons (prussic acid) disappear from certain plants when they are grown in darkness, are given as evidence to support the hypothesis that toxic ingredients of plants are normal products encountered in the processes of metabolism. These processes are more active in the growing than in the maturing plant, and in the plant kept in darkness photosynthetic processes are reduced to a minimum.

VII. DETECTION OF POISONOUS PLANTS AND THEIR ACTIVE INGREDIENTS IN THE BODY.

A. In the Gastro-intestinal Tract.

The examination of the stomach (rumen in ruminants) contents is of great value in peracute and acute plant poisoning as in such cases it is quite likely that remains of the plants ingested will still be found in the stomach. It is, however, in most cases not an easy matter to identify with any amount of certainty the masticated and partly digested portions of plants present in the stomach. Leathery leaves and hard fruits (seeds) will retain their original appearance to a far greater extent than those more easily digested. In the case of poisonous plants which exert their effects comparatively long after ingestion (*Pachystigma pygmaea* Schltr. Robyns, *Crotalaria dura* Wood and Evans, *Crotalaria globifera* E. Mey., and species of *Senecio*), the examination of gastric contents is of relatively little value as the animals may die weeks and even months after the plant has been eaten. On the other hand, portions of the leaves of *Dichapetalum cymosum* Hook (gifblaar) and species of *Homeria* and *Moraea* (tulips) may be found in the stomach contents of animals which have succumbed within a short while after feeding on these plants.

During life the faeces, milk and urine may be examined for the presence of any poisonous substance that is suspected. A poison may find its way into the faeces through being incompletely absorbed from the gastro-intestinal tract, or/and through excretion by the bile and gastro-intestinal mucosa.

B. In the Blood and Organs.

The methods of detection of poisons in the blood and organs cannot be so extensively applied to plant poisons as in the case of mineral poisons owing to the fact that our knowledge of the poisonous ingredients of plants, especially those indigenous to South Africa, is very limited. Very little is known of the active principles of South African poisonous plants, their distribution in the body, their extraction from the blood and organs, and their identification by chemical and pharmacological means.

I need hardly stress the point that the utmost care should be exercised in drawing conclusions from toxic substances that have been isolated from the gastro-intestinal contents, as these contents may harbour poisonous substances (for example biogenic amines), which pharmacologically may have effects on animal organs very similar to those exerted by the active principles of certain plants. The longer the period between death and the examination of the gastro-intestinal contents the greater is this danger owing to processes of decomposition.

The mere presence of a poisonous substance in an organ or organs by no means warrants a diagnosis of poisoning by such a substance, this evidence being corroborative and not diagnostic. The amount of poison present in an organ, the nature of this organ, the amount of poison ingested and the time that has elapsed between ingestion and death, are of the utmost importance in the diagnosis of poisoning.

The amount of poison present in the gastro-intestinal tract is not of such great importance in the diagnosis as the quantity present in organs to which this poison has been conveyed by the blood. Fatal quantities of poisons may be present in the gastro-intestinal contents without causing symptoms of poisoning [that is, when they are present in an inabsorbable (insoluble) form] whilst the presence of any such poison in the organs is of great diagnostic value.

The amount of poison present in an organ depends on the quantity ingested and on the period of time that has elapsed since ingestion. It is quite possible that a poison, when taken in a small quantity over prolonged periods, may cause damage to an organ or organs, and that this damage progresses after all traces of the poison have been eliminated from the body. This probably is the case in poisoning by *Crotalaria dura* Wood and Evans and in *Pachystigma pygmaea* Schltr. Robyns, as animals which have partaken of fatal amounts of these plants may only die from "Jaagsiekte" or "Gousiekte" months after they have eaten the plants.

The knowledge of the distribution of a poison in the body is essential in the collection of specimens for analysis. A trace of prussic acid in the brain is of far greater significance than its detection in the gastric contents, and in carbon monoxide poisoning no co-haemoglobin is to be found in the blood of the spleen and bone-marrow. In chronic arsenical poisoning, which occurred months before the investigation, arsenic will be found in the hoofs, claws or nails and hair of the victims and not in the liver, kidneys and gastro-intestinal contents as in acute arsenical poisoning. As a rule the most essential organs to be collected in cases of suspected plant poisoning are liver, kidneys and gastro-intestinal wall, as well as gastro-intestinal contents and urine.

It should also be mentioned here that antibodies will be detectable in the blood of individuals who have partaken of plants containing toxalbumins provided such individuals live for such a period as to allow of the development of such antibodies.

VIII. DIAGNOSIS OF PLANT POISONING.

In addition to what has been said under "Detection of Poisonous Plants and of their active ingredients in the body", the following remarks may be made here:—

It is in many cases a very difficult task to make a definite diagnosis of poisoning. The following method of procedure is adopted by the author in the investigation of suspected cases of poisoning.

A. Anamnesis.

The *anamnesis* is of the utmost importance in poisoning, especially information concerning the feeding or grazing of the animal or animals concerned prior to the development of symptoms of poisoning. The fact that animals may develop symptoms of poisoning months after they have eaten a particular plant (*Pachystigma pygmaea* Schltr. Robyns), must be borne in mind.

B. Symptomatology.

A sudden onset of symptoms of disease, especially when several animals are affected at the same time, is indicative of poisoning. Again, symptoms like profuse diarrhœa, skin lesions (photodermatitis), inappetence, icterus, disturbances of the heart action, respiration, urinary secretion and central nervous system (convulsions, staggering, shivering, pushing, wandering about aimlessly, delirium, hallucinations, chorea, tetany, hyperaesthesia, depression, blindness, paralysis and coma), may strengthen this suspicion. Some cases of plant poisoning may resemble the course taken by infectious diseases in that they have a period of latency (incubation period) and cause the development of fever. The point of greatest importance in distinguishing between infectious diseases and cases of plant poisoning, when they make their appearance in herds or flocks, is that, as a rule, in the former case one or only a few animals will be suddenly taken ill, whilst in the latter case a large percentage of a herd or flock will develop symptoms of disease at the same time. Sometimes symptoms characteristic of certain poisonous plants assist us in diagnosing such cases (tribulosis, vangueriosis, alopecia, seneciosis).

C. Post-mortem Appearances.

These are of great value in the differential diagnosis as the lesions found in many diseases and those causes by poisonous plants are known.

D. Histology.

Also histological lesions may be of diagnostic value, e.g. cirrhosis of the liver in seneciosis; fibrosis of the myocard in " gousiekte ".

E. Examination of Gastro-intestinal Contents.

In many cases of peracute and acute plant poisoning the remains of masticated and partly digested portions of the responsible plants may be found in the stomach (rumen in ruminants).

F. Isolation of the Active Principles of Plants from the Gastro-intestinal Contents and Organs and their Chemical and Pharmacological Identification.

In this connection our knowledge of South African plant poisons is very limited indeed and hence of comparatively little value in the diagnosis of plant poisoning.

It should, however, be pointed out that the utmost care must be exercised in expressing an opinion as to the nature of poisons, especially when only traces are present, in corpses and carcases, because it should be remembered that poisons like ptomaines, markedly resemble some vegetable poisons. Aconite-like, coniine-like, codeine-like, colchicine-like, veratrine-like, and strychnine-like ptomaines are known (Autenrieth, 1928). It is, therefore, most essential that wherever possible chemical tests for poisons should be confirmed by biological tests. To my mind, a positive biological test for strychnine is absolutely essential before it can be definitely stated that strychnine is present in a corpse or carcass.

IX. PROGNOSIS OF PLANT POISONING.

The prognosis depends on a large number of factors, which are fully discussed under "Factors concerned in the Determination of the Toxicity of Plants". Suffice it to say here that in most cases of poisoning it is very difficult to predict the result not only with regard to death, but also as far as complete recovery is concerned. Furthermore, some plants exert their effects in a two-fold manner as in the case of poisoning with *Adenia digitata* (Harv.) Harms, and improvement after exhibition of severe symptoms of poisoning is by no means an indication that the patient is well and on the way to recovery. The tuber of this plant contains two poisonous principles prussic acid and a toxalbumin, modeccin. Symptoms of prussic acid poisoning set in very soon after ingestion of the tuber. The victim may, however, survive, and may only succumb to the effects of the toxalbumin, modeccin, which exerts its actions on the system within a few days after ingestion.

In the case of plants which exert their effects only after a certain period of latency, it is an even more difficult task to express an opinion as to the future health of such an animal. The sooner after ingestion of poisonous plants the animals are treated, the more favourable the prognosis will be, as the sooner the bowels are emptied the smaller the quantity of poison absorbed.

X. GENERAL PRINCIPLES OF TREATMENT OF PLANT POISONING.

The general principles of treatment of all cases of poisoning are embodied in the following rules: (A) Prevention of further absorption of the ingested poison, (B) treatment of symptoms of poisoning, and (C) promotion of excretion of the poison. In addition, the animals should be prevented from further ingestion of the poison.

(A) Prevention of Further Absorption of Poison.

The most effective method of procedure as adopted by the author is (*a*) to prevent the animals from drinking water, and (*b*) to render the poison still present in the gastro-intestinal tract inabsorbable and to remove it by the administration of purgatives on emetics or by stomach lavage. The absorption of poison still present in the gastro-intestinal canal may be retarded or prevented by administering chemical and physical antidotes. Tannic acid will, for example, cause the precipitation of insoluble alkaloidal tannates, and will therefore be of great value in preventing the absorption of poisonous alkaloids. Potassium permanganate will cause the destruction of many plant poisons through oxidation; it is said to be the most effective when in acid solution. Furthermore, some poisons exert their toxic effects in an acid environment, whilst others require an alkaline medium. Animal and wood charcoal and liquid paraffin may be used as physical antidotes. Charcoal is an active absorber of many plant and mineral poisons, and liquid paraffin, being absorbable to only a very slight extent, will when passing through the gastro-intestinal canal, carry with it a certain amount of the

poison present. When the administration of these two substances is followed by purgatives, the amount of poison absorbed can be appreciably reduced. The absorption of poisons from the gastro-intestinal tract can be further retarded by the administration of astringents (tannic acid, lime water, bismuth subnitrate, alum). It goes without saying that the purgatives to be used in cases of poisoning must act quickly, and whenever possible purgatives which require water as a solvent must be avoided, as in many cases the introduction of water will facilitate absorption of the poison. In equines arecoline, pilocarpine and eserine, administered subcutaneously, are valuable purgatives, whilst in cattle, sheep, goats, pigs, dogs, cats and birds calomel, castor oil and croton oil must be resorted to in the first place. It is not advisable to use saline purgatives in cases of plant poisoning for the reasons mentioned above.

As emetics the following may be used: Apomorphine, veratrine rhizoma veratri albi, radix Ipecacuanhae, tartar emetic, zinc sulphate, common salt and mustard. Emetics are to be used only in those animals which vomit with ease.

In the irrigation of the stomach it is essential that substances, which are likely to form insoluble compounds with the poison, be used. When plants containing alkaloids as the active principle cause poisoning, then tannic acid should be used, as it forms insoluble alkaloidal tannates and at the same time is an astringent, thus retarding absorption. Furthermore, glucose should be administered to animals in all cases of poisoning, especially where liver damage occurs, as the detoxicating effect of this organ appears to be directly dependent on its carbohydrate content.

(B) Treatment of Symptoms of Poisoning.

In most cases we have to resort to the treatment of the symptoms as they arise (symptomatic treatment), as very few specific antidotes, which will inactivate the poison in the blood stream and in the organs, are known. Cramps and convulsions are traeted with sedatives and narcotics and symptoms of paralysis with stimulants (strychnine), whilst in prussic acid poisoning we have a specific antidote in some sulphur preparations forming the harmless sulphocyanides. The administration of heart and respiratory stimulants is essential in many cases of plant poisoning.

Gastro-intestinal irritation may in many cases be effectively treated with raw linseed oil or limewater, either alone or mixed in equal parts. The author found that the beneficial effect of this mixture in the treatment of obstinate diarrhœa may be appreciably increased by the addition of a small amount of tannic acid. Other substances that have an alleviating effect on gastro-intestinal irritation are linseed and barley gruel and the whites of eggs beaten up in milk.

Other essential points in the treatment of poisoned animals is to shade them and to allow them as much rest as possible. Driving, especially in cases of animals suffering from poisoning with plants which affect the central nervous system (cynanchosis, cotyledonosis,

equisetosis), in many instances causes death, while it left undisturbed the animals stand a better chance of recovering. It is also essential that poisoned animals should receive a suitable diet.

(C) Promotion of Excretion of the Poison.

Excretion of poisons may be facilitated by the administration of purgatives, diuretics, cholagogues, sialagogues, and diaphoretics. Many purgatives stimulate the secretion of the glands of the gastro-intestinal mucosa, which excretes many poisons, and, in addition, such drugs prevent, to a certain extent, the reabsorption of bile, which carries with it poisons excreted by the liver, by causing a quick passage of the intestinal contents. The kidneys are the most important organs concerned in the excretion of poisons, hence the effect of stimulating renal secretion by means of diuretics is clear. Also the liver, salivary glands and sweat glands are active excretors of many poisons. The fact that lactating mammary glands are very active excretors of poisons, should be borne in mind, as cases of poisoning in human beings may arise from drinking the milk of poisoned animals.

The bleeding of animals suffering from poisoning is considered by some authorities to be of no value, but it seems likely that much benefit will be derived from bleeding affected animals and replacing the volume of blood lost by physiological saline solution containing calcium gluconate or by blood transfusion. Through the bleeding a certain proportion of the poison contained in the blood is removed from the body, calcium in many cases of poisoning supports the fighting powers of the body against poisons, and it has been established that the higher the glycogen content of the liver the more effective it is as a detoxicator.

In many cases of poisoning, especially when poisons causing haemolysis and reduction in the oxygen carrying capacity of the blood are concerned, blood transfusions are of life-saving value.

XI. FACTORS CONCERNED IN THE DETERMINATION OF THE TOXICITY OF PLANTS.

A. The Plant.

The most important factor concerned in the determination of the effects of a poisonous plant on any subject is the rate at which such a plant is ingested. Most striking examples of how the toxic and lethal doses of poisonous plants can vary are found amongst those plants which contain gaseous or volatile poisons which are rapidly eliminated. An animal may, for example, eat with impunity within twenty-four hours a certain quantity of a plant containing prussic acid without suffering any ill-effects, while the same quantity, if eaten within six hours, would cause death. The degree of toxicity of a plant, therefore, depends on the time allowed to the body tissues and fluids to eliminate or to destroy the poisonous principle.

It is well known that individual plants of the same species growing in different localities vary to a considerable degree in their active principle content. At Onderstepoort, Steyn (1932) found that specimens of **Cotyledon leucophylla* C.A.Sm. in the same stage of development and growing beside one another varied considerably in toxicity. Soil and climatic conditions are to a very large extent responsible for the difference in toxicity, but this cannot account for the differences in toxicity of plants of the same species growing beside each other. The latter point is discussed under " Transmission of the Degree of Toxicity of Progeny ".

In spite of the fact that much time has been spent in the investigation of factors thought to be concerned in the determination of the toxicity of plants, no definite results have as yet been achieved. It is impossible here to discuss at any great length, and the following factors, which may play a rôle in the production of poison in plants, will be briefly discussed.

(*a*) *Soil and cultivation.*—The composition, character, bacterial and protozoal content, moisture content and temperature of the soil probably, in some cases at least, influence the production of poison in plants. The results of experiments with fertilizers are so variable that no definite conclusions can be drawn, except in the case of nitrates, which increase the prussic acid content of plants (Burtt-Davy, 1912; Couch, 1932). It must be mentioned that fertilizers are extensively used in the growing of medicinal plants in order to stimulate growth and thus increase the amount of active principle per unit area of soil surface. This use of fertilizers has, however, no relation to the utilization of fertilizers as far as the increase of active principle content per unit weight of plant is concerned. The character of the soil may possibly have an influence on the toxicity of plants, as the more porous a soil the better the aeration, and consequently oxidation and other processes dependent upon a free oxygen supply will be more active than in less porous soils. The moisture content and temperature, as well as the oxygen content of the soil are bound to influence the microbiological processes in the soil to a considerable extent. These varying conditions in the soil may be, and probably are, responsible for the production of substances of a very variable nature in soils of a different character, and these substances may in turn affect the toxicity of plants. Many farmers hold that *Pachystigma pygmaea* (Schltr.) Robyns (gousiekte bossie) growing on black clay soil, is much less poisonous than when growing on red sandy soil. It is also maintained that *Geigeria passerinoides* Harv. is most toxic when growing on limestone. In experiments conducted at Onderstepoort with *Cotyledon orbiculata* L. the specimens grown on black clay soil were less toxic after one year than at the time the experiment was begun, whi lthe reverse was the case with those specimens grown on red sandy soil (Steyn, 1932).

Cultivation produces a decrease in the toxicity and even the complete disappearance of the toxic constituents of some plants, whilst it apparently has no effect on the toxicity of other plants.

*** *C. orbiculata* Burtt-Davy, A. Trans. & Swaz., I. 142, 143 (1925); ext. ref. Bot. Mag. et Journ. Bot. et extra Tvl. cit.; non Linn. (1753).**

The seeds of cultivated varieties of *Phaseolus lunatus* L. constitute a valuable foodstuff, whereas those of the wild varieties contain fatal amounts of prussic acid. In cultivation there are improved conditions in connection with irrigation, aeration of the soil, and fertilization, and these probably influence the degree of toxicity of some plants. On the other hand, cultivation appears to have no noticeable effect on the toxicity of *Nerium oleander* L. (Morrison, 1926).

(*b*) *Climatic conditions.*—Temperature and moisture content of the air will to a large extent influence, as explained in (*a*), the chemical and microbiological processes in the soil. The alkaloidal content of medicinal plants growing in the same locality was found to be at its lowest in years of low temperature and deficient sunshine. *Ephedra* spp. grown in areas with a high rainfall are less toxic than those grown in localities with a low rainfall. Furthermore, these plants were found less toxic in rainy than in dry years (Ghosh and Krisha, 1930).

(*c*) *Nature and intensity of light.*—The nature and intensity of light depend on the time of the day, the altitude and the degree of cloudiness. The alkaloidal content of *Atropa belladonna* L. could not be increased by means of fertilizers, but full exposure to sunlight appeared to increase its active principle content (Ransom and Henderson, 1912), and Burman (1911) states that the alkaloidal content of this plant is at its lowest in years of low temperature and deficient sunshine. It appears that certain species of *Cotyledon* (*Cotyledon ventricosa* Burm., *Cotyledon wallichii* Harv.) are not dependent on the direct rays of the sun for the production of poison as they are almost invariably found growing under bushes and shrubs. It is, however, quite possible that some plants are to a certain extent dependent upon photosynthetic processes for the production of poison and that they do require light in the elaboration of poison Treub (Robinson, 1930) found that the darkening of the plant "*Pangium edule*" causes a decrease in its prussic acid content. It was found that plants of *Datura Stramonium* L. kept on a balcony facing east, south-east had a higher active principle content than those kept on a balcony facing north-west. Possibly certain rays of the visible and invisible spectra are essential to stimulate the production of poison.

(*d*) *Season.*—The poisoning of stock grazing under natural conditions, is most prevalent during the spring months. This is due not only to the fact that many poisonous plants are very deeprooted (*Dichapetalum cymosum* Hook) or are of a bulbous nature and hence are not dependent on spring rains for the development of young leaves and shoots, but also to the fact that many plants are most poisonous in the young stage of development. The active principle content of many plants varies in amount not only at different times of the year, but also at different hours of the day. André (Czapek, 1921) found the oxalate content of *Mesembrianthemum crystallinum* L. to be about five times higher in May than it was in August, whilst the reverse was the case with the malic acid content of this plant. Baur (Hecht, 1931) found that the percentage of active principle contained in *Atropa belladonna* L. and *Datura stramonium* L. was at its highest in the early morning hours. The early development of the leaves

and flowers of deeprooted and bulbous plants constitutes a great temptation to animals when the remaining vegetation is still dry and unattractive. It is for the same reason that so many animals succumb to plant poisoning during periods of drought.

(*e*) *Stage of development.*—Many plants may contain fatal amounts of poison when immature, but when mature they constitute valuable foodstuffs for man and animal. The unripe seeds of *Linum usitatissimum* L. may contain fatal amounts of the cyanogenetic glucoside, linamarin, whilst the ripe seeds in the form of cakes form a valuable foodstuff for stock. The wilted and stunted plants of *Zea mays* L., various species of *Sorghum* and certain other grasses contain large amounts of prussic acid, the younger the stage of growth, the higher the prussic acid content. An outstanding example of plants that are most toxic in the early stages of development is *Dichapetalum cymosum* Hook (gifblaar), in which the young leaves are much more toxic than the older ones. On the other hand, the young and mature leaves of *Cotyledon orbiculata* L. are equally toxic. The ripe berries of *Melia azedarach* L. are more toxic than the immature ones, whilst the berries of *Solanum nigrum* L. and *Solanum incanum* L. are poisonous when green and harmless when ripe.

(*f*) *Toxic parts of plants.*—The distribution of poisonous substances in plants in the same stages of development varies with almost every species of plant. The following plants may be quoted as examples: the bark and wood of the roots of *Derris elliptica* Benth are very poisonous, whilst the stems are slightly poisonous and the leaves not at all; the leaves of *Taxus baccata* L. (the yew tree) are more toxic than the fruit and it is said that the male tree is slightly more toxic than the female: the drupes of *Melia azedarach* L. are much more poisonous than the leaves; while in the case of *Datura stramonium* L. the seed contains less active principle than the leaves, stems and roots. Not only may the different parts of a plant vary in the amount of active principle they contain but the various portions of the same part may also vary to a considerable extent. Some peach, apricot and prune kernels contain fatal amounts of prussic acid, whilst the outer portion of the fruits are extensively eaten. Some plants contain their active constituents in a form other than that which exerts its poisonous effects on the body. Cyanogenetic glucosides, as such, are non-poisonous and require enzyme- or acid-action in order to liberate the deadly prussic acid.

(*g*) *State of plants.*—Some plants when dried and stored rapidly decrease in toxicity whilst others retain their active principle content for very long periods. The former will be the case especially with those plants which have gaseous (pussic acid) or volatile (ethereal oils) active principles. The active principles of other plants may, however, be destroyed by chemical processes (dehydration, oxidation, hydrolysis, ferment action, etc.), which continue or may set in after the plants have been collected. It is self evident that plants which are to be tested for the presence of any poisonous constituents formed in them under natural conditions, should not be in a decomposed state or attacked by fungi as both the decomposed matter and the fungi may of themselves liberate poisonous substances.

(*h*) *Transmission of the degree of toxicity to progeny.*—Certain plants are capable of passing on their degree of toxicity to their progeny. This has been proved to be the case with *Atropa belladonna* L. and *Papaver somniferum* L. (Hecht, 1931). Steyn (1932) pointed out that reliable results in connection with the investigation of the factors concerned in the determination of the toxicity of plants can hardly be expected when any one experiment is conducted with a large number of plants at the same time. This point came to the author's notice while conducting experiments with *Cotyledon orbiculata* L. Specimens of this plant in the same stage of development and growing beside one another were found to differ in toxicity to a considerable degree; also one and the same plant varied considerably in toxicity within short periods. It is because of these two facts that it is essential to conduct experiments with one and the same plant. Unfortunately the size and nature of many poisonous and medicinal plants do not allow of this procedure. In such cases where fields of plants have to be used in any one experiment at the same time, it would be of great importance to use the progeny of one and the same plant as, according to experiments conducted with *Atropa belladonna* L. and *Papaver somniferum* L. the toxicity of such plants will be approximately the same as that of the parent plant. *Cotyledon orbiculata* L. is eminently suited for investigations as to the influence of various factors on its toxicity as the nature of this plant is such that it can be used in one and the same experiment over a period of years. The procedure adopted at Onderstepoort is to follow the course of the toxicity of individuals of this species for one year and then submit some individuals to various influences and again follow their course of toxicity. In addition control specimens are grown and the course of their toxicity followed. It is hoped that by following this procedure we will be able in the course of time to throw some light on the factors influencing the production of poison in plants.

(*i*) *Nature of toxic ingredients of plants and channel of introduction into the body.*—The factors determining the degree of toxicity of a poison are the following: (1) Its rate of absorption. Gaseous and volatile poisons are most easily absorbed owing to their high degree of diffusibility. Poisons in solution or in a state of very fine division will act on the body more markedly as they are more rapidly absorbed, thus leaving little time for elimination or inactivation. For the same reason the toxicity of any poison is directly also plays an important rôle in the rate of absorption. It is evident that the larger the surface of absorption, the more quickly will the poison be absorbed. (2) The rapidity of its inactivation in the body. (See "The Fate of Plant Poisons in the Body".) Vomicin, an alkaloid contained in *Strychnos Nux Vomica* L., is not poisonous when taken per os or injected subcutaneously, but only when introduced directly into the blood stream, as it is very rapidly inactivated in the tissues. (3) The rate of elimination. The more rapid the elimination of a poison from the body the less likely will it be to cause severe damage to the tissues. The highest degree of damage will be caused by a poison which has a special affinity for, or, is retained by a certain organ as its elimination will be retarded, with the result that it may exert cumulative effects. Gaseous and

volatile poisons are amongst those which are most rapidly eliminated. (4) The mode of application. The channel of absorption is an important factor in determining the degree of toxicity of a poison (see "Absorption of Poisons"). Many poisons act most rapidly when introduced directly into the blood stream, whilst others are inactive when introduced by this channel and require to be acted upon by the gastro-intestinal juices. The latter is the case with cyanogenetic glucosides, which are non-poisonous when introduced parenterally and which, when subjected to ferment or acid action in the stomach, liberate prussic acid. The same applies to mustard oil compounds, which require to be acted upon by ferments in order to liberate the active mustard oil. Croton glycerine, contained in the seeds of *Croton tiglium* L., is non-poisonous when introduced parenterally, as the gastric juice is required for the liberation of the active crotonic acid. Curare, a South American arrow poison, is absorbed slowly when taken by the mouth and is rapidly eliminated after absorption, hence it is comparatively speaking, non-poisonous when introduced by this channel. (5) Some plants require the presence of yet another plant in the gastro-intestinal tract in order to liberate its active principle in an active form. Plants may contain cyanogenetic glucosides, which require ferments, which are present in other plants, for the liberation of prussic acid. This is probably the case with *Eremophila maculata* F.M., whose cyanogenetic glucoside requires for its decomposition a ferment contained in *Acacia Georgina* Bailey (Finnemore and Cox, 1927). It should also be mentioned here that a plant may contain the pharmacological antagonist of its active principle. *Digitalis purpurea* L. for example, contains digitonin, which when administered intravenously is a pharmacological antidote to digitoxin.

From the above it is clear that when discussing the toxicity or harmlessness of a plant the following points should be mentioned, (1) origin of the plant, (2) part of the plant tested, (3) state of plant (whether fresh, wilted or dry), (4) stage of growth, (5) experimental animal used, (6) method of administration of plant (eaten or administered), and (8) the period in which the plant was eaten or administered.

Fodder plants may be rendered poisonous when attacked by fungi or decomposed by bacteria or ferments (see Fungi in Relation to Health in Man and Animal). Certain processes to which foodstuffs are subjected in the course of their preparation may also be the cause of poisoning by such foodstuffs (see "Poisonous Foodstuffs").

B. The Animal.

It is well known that the toxic or minimum lethal dose of a poison may vary to a considerable extent in human beings, in animals belonging to different classes, and also in animals of the same class. In the course of experiments conducted at Onderstepoort it was found that the degree of resistance of the same rabbit to the same dose of potassium cyanide varied considerably from day to day (Steyn, 1932b). One or more of the following factors may be responsible for this variation in susceptibility.

(*a*) *Species of animal.*—The various species of animals as a rule vary in their susceptibility to poisons. This variation may in some cases be explained on physiological and anatomical grounds. There is for example, a great difference in the method of preparation of plants for digestion and absorption as well as in the anatomical structure of the gastro-intestinal tract of ruminants and non ruminants. As a rule poisonous plants after ingestion will act more quickly in non-ruminants than in ruminant as the latter poison is much more diluted in the stomach and the passage of the ingested plants from the stomachs into the small intestine is much slower than in non-ruminants. The active ferment and bacterial action to which poisonous plants are exposed in the rumen may to some extent destroy their active principles. Canines and fowls use very little or no saliva in swallowing their food, whilst cattle, sheep, and horses, during the process of mastication, secrete enormous amounts of alkaline saliva. This alkaline saliva may destroy the actions of such active principles of plants which require an acid medium in order to exert their actions. Such acidophilous active principles will, on the other hand, have pronounced effects on the dog, in which animals this protecting influence of the saliva is absent.

Many examples of the difference in the susceptibility of the different classes of animals to the same plant poison may be mentioned. The following are the most outstanding: Horses are much more susceptible than cattle to poisoning by ricin and *Ornithogalum thyrsoides* Jacq; sheep and goats are very resistant to the ripe drupes of *Melia azedarach* L. (syringa berry), whilst pigs are very susceptible; and the quantities of some species of *Senecio*, which will cause death in dogs, has no effect on rabbits. In addition to this difference in susceptibility of animals to the same poison, many poisons produce different symptoms in animals belonging to different classes of animals, as is illustrated by the following examples: *Crotalaria burkeana* Benth, which affects the hoofs in cattle, causes staggers in horses; *Crotalaria dura* Wood and Evans causes catarrhal gastro-enteritis and cirrhosis of the liver in cattle, whilst in horses and sheep it usually affects the lungs; several species of *Geigeria* usually cause vomiting and other gastro-intestinal disturbances in sheep and goats, while in cattle it frequently produces paralysis; and morphine has a sedative effect on dogs but is a powerful nervous stimulant in cattle.

This difference in susceptibility to poisons is sometimes met with in the same class of animal. Ducks and geese are for example, about ten times more resistant to prussic acid than fowls are (Forchheimer, 1931).

(*b*) *Breed of animal.*—Highly bred animals are, as a rule, more susceptible to poisons than mixed breeds. The general resistance of the former animals has been probably reduced by methods of breeding and by being kept under artificial conditions. A thoroughbred is known to be more susceptible than an ordinary cart horse to the actions of poisons. The same applies to purebred cattle and native cattle. Experiments conducted with potassium cyanide upon white Angora rabbits, ordinary short-haired white rabbits and grey rabbits,

proved the former more susceptible than the two latter breeds (Steyn, 1932b). This phenomenon is most probably due to the selective breeding of Angora rabbits.

(c) *Size of animal.*—The toxic or lethal dose of a poison is determined per unit body-weight of animals, as the size and weight of animals belonging to the same class may vary to a considerable extent, especially in the case of dogs and horses. Vollmer (1931) found that small mice are less susceptible to alcohol than larger mice. Care must be exercised in the choice of experimental animals used to determine the relative toxicity of poisons, as in the case of long woolled or pregnant animals we cannot calculate the minimum lethal dose per unit body-weight. Some poisons are more toxic to small than to larger sized animals of the same class. Colchicin and hydrochinon, whose oxidation products are poisonous, are more deadly to small than to large sized mice and rats, whilst in ethyl alcohol which is detoxicated by oxidation, the reverse is true. Vollmer (1932) holds these phenomena to be proof that the degree of oxidation is higher in small than in large animals. It therefore appears essential that in the determination of the minimum lethal dose animals of approximately the same size and condition should be used. Animals with a high percentage of body fat will, when the dose is calculated per unit body weight, receive relatively higher doses than animals in normal condition. This naturally will give rise to wrong calculations as to the minimum lethal dose per unit body weight. Body fat, which has very little or no value in the detoxication of poisons in the body, places such an animal at a disadvantage if it ingests an amount of poison calculated on the minimum lethal dose per unit body weight.

(d) *Age of animal.*—Generally speaking, the younger the animal the more susceptable it is to the actions of poisons. There are, however, exceptions. Young rabbits are more resistant to strychnine than full grown ones, and puppies in their first and second month require more apomorphine to produce vomiting than adult dogs (Schlossmann, 1931). Young dogs withstand bigger doses of calomel than full grown ones, whilst the former are much more susceptible to santonin than the latter. Animals in extreme age are more susceptible to poisons than animals in the prime of life. This phenomenon is due, partly at least, to the impaired functions of the organs of excretion and of those organs, tissues and fluids, which play a rôle in protecting the system against the action of poisons.

(e) *Condition of animal.*—Animals in a bad condition or suffering from some or other disease are more likely to be affected by poisons than animals in good condition and in good health. The general resistance of the former animals are lowered and in addition poisonous plants may attack organs which are already affected by disease. On the other hand, it was found that patients suffering from strychnine poisoning and from tetanus, stand three or four times the dose of chloral which will be tolerated by an ordinary human being (Smith, 1932).

Animals with diseased organs of excretion will naturally have an increased susceptibility to the action of poisons. Inflammatory conditions in the gastro-intestinal tract, which increases the permeability

of the mucosa, facilitate the absorption of poisons. Worms which damage the intestinal mucosa will also accelerate absorption of poison from the gastro-intestinal canal. The detoxicating effect of the liver, which is of great importance in rendering poisons inactive, is greatly reduced by cirrhosis caused by liver fluke. Non-parasitic and parasitic skin diseases which destroy the protecting effect of the epidermis will naturally facilitate the absorption of poisons from the skin. Such cases of poisoning with dipping fluids frequently occur. It is, however, interesting to note that Leischman patients, as a rule, tolerate doses of tartar emetic better than individuals in good health, as antimony has a greater affinity for the Leischman parasites than for the various body tissues.

(*f*) *Sex of animals.*—It is probable that pregnant animals (and the more advanced the state of pregnancy the greater the probability) will be more susceptible to poisons than non-pregnant ones. It is not an easy task to compare the relative susceptibility of pregnant and non-pregnant animals to poisons as it is impossible to know the weight of the contents of the uterus, which should be deducted from the weight of the pregnant animal in calculating the minimal lethal dose per unit body weight. Females are, generally speaking, more susceptible than males to poisons. It is, however, quite possible that females in the lactation period may tolerate poisons better than males, as the lactating udder is an active excretor of poisons. On the other hand, lactating animals may possess an increased susceptibility to some poisons. This is the case with poisons such as oxalic acid and oxalates, which decrease the diffusibility of the blood calcium; hence lactating animals, which excrete large amounts of calcium in the milk, will be more susceptible than others. The same applies to cases of lead poisoning, as calcium facilitates the fixation of lead in the tissues.

(*g*) *Colour of animal.*—The colour of an animal plays a most important rôle in skin diseases caused by photosensitization, the unpigmented skin exposed to the direct sunlight being the only part to suffer. Those portions of the skin which are pigmented or well protected by wool or hair, are not affected. Plants responsible for photosensitization are *Tribulus terrestris* L., *Trifolium* spp. and *Hypericum aethiopicum* Thunb. to mention but a few.

(*h*) *Temperature.*—Extremes of temperature lower the body resistance and antagonise the elimination of poison by decreasing secretion. In experiments on frogs and cats Hirschfelder and his collaborators (1920) found that the toxic action of digitalis increased with elevation of the body temperature. This phenomenon is of the utmost importance in the calculation of doses of drugs for patients. The higher the body temperature the quicker the heart action, with the result that more poison will be absorbed and pass through the organs and tissues per unit time. This fact, to some extent at least, is responsible for the phenomenon that poisons are more active on hot days or when the affected individuals exert themselves. For the above reason it is quite clear why the hot sun has a detrimental effect on poisoned animals.

(*i*) *Exertion.*—Exertion will effect increased heart action and temperature, hence will render subjects more susceptible to the action of poisons for reasons mentioned under (*h*). In addition, excitement will have a detrimental effect on the condition of poisoned subjects, especially those in which the central nervous system is affected. It is well known that driving animals suffering from *Equisetum ramosissimum* Desf. and *Cotyledon* poisoning will have fatal effects, whilst many animals will recover if left undisturbed.

(*j*) *Seasonal variation in susceptibility of animals.*—Armitage and his collaborators (1932) state that the blood pressure in cats is higher in spring than in winter, probably due to an increased activity of the sympathetic part of the autonomic nervous system. The nervous system is said to be more sensitive during spring. Hogden (1931) found a seasonal variation in the amount of calcium in the blood of the South African toad. It is quite conceivable that animals will be less susceptible to the actions of poisons and drugs in winter than in summer for reasons mentioned under (*h*). Hunt (1910) in his summary says: " Season has an important effect upon the resistance of animals to certain poisons; in some cases these effects seem to depend upon seasonable variations in the activity of the thyroid ".

(*k*) "*Conditioned reflexes.*"—Pavlov and Krylow (Editorial, 1930) conducted some most interesting experiments upon dogs with apomorphine and morphine. Dogs were given small amounts of apomorphine subcutaneously and at the time of injection a note of a certain pitch was sounded. After having repeated these injections several times, salivation and vomiting could be produced by the sound of the note alone. After dogs had received morphine injections on several days, salivation, nausea, vomiting, followed by profound sleep, could be caused to develop by allowing them to watch only the preparation for the injection. Such morphine treated dogs sometimes exhibited symptoms of morphine poisoning when seeing the experimenter or after the injection of a harmless fluid.

(*l*) *Conditions which favour or impede dissolution and absorption of, or, which effect changes in poisons present in the gastro-intestinal tract.*—(1) *Water.*—One of the most essential points in the treatment of animals suffering from plant poisoning is to withhold water from such animals until all the ingested plant material is removed from the gastro-intestinal tract. It stands to reason that water will facilitate the dissolution and absorption of the active principles of the ingested plants, especially those very soluble in water. Animals poisoned by *Diacheptalum cymosum* Hook, whose active principle is very soluble in water, frequently drop down dead soon after drinking water.

(2) *Drugs.*—Certain drugs when present in the gastro-intestinal tract, organs or body fluids, will protect the system against certain poisons. This is the main principle upon which we base preventive and remedial treatment of cases of poisoning. In South Africa great success has been achieved in the prevention of prussic acid poisoning in sheep resulting from the ingestion of certain grasses in a wilted state by supplying these animals with licks containing sulphur (see **Gramineae**). Acids introduced into the gastro-intestinal tract will

facilitate the liberation of prussic acid from cyanogenetic glucosides. Macht and Finesilver (1922) found that sodium sulphate and magnesium sulphate markedly retard the absorption of phenolsulphonphthalein, potassium cyanide, chloretone, apomorphine, morphine, pantopon, cocaine, hydrochloride, strychnine, quinidin, tincture of digitalis, sodium salicylate, salol, aspirin, antipyrin, potassium iodide, urotropine, corrosive sublimate and phenol. Calomel, cascara sagrada, and castor oil did not have this effect on the above-mentioned drugs. Under " General Principles of Treatment of Plant Poisoning " the author has not recommended the use of saline purgatives on account of the relatively large quantities of water that are used to dissolve these purgatives. It is advisable to uphold this recommendation in the treatment of animals poisoned by South African plants as the active principles of most of these plants are unknown with the result that it is not definitely known whether saline purgatives will retard their absorption. Kohn and Costopanagiotis (1932) found that a 10 per cent. solution of urea injected intravenously decreased the toxicity of digitalis by 30-40 per cent.

(3) *Substances normally present in the gastro-intestinal juices.*—Acid, bacterial, ferment and enzyme action in the gastro-intestinal tract may facilitate or retard the liberation, dissolution or absorption of poisons. With regard to the rôle played by bile in the absorption of poisons, Langenecker (1930) says, that it facilitates absorption by increasing solubility, by decreasing absorption to charcoal and by rendering the cell wall more permeable.

(*m*) *Diet.*—The diet of an animal may have a pronounced effect on its susceptibility to the effects of poisons as is illustrated by the following examples: Hunt (1910) in his summary writes: " (1) A restricted diet markedly increases the resistance of certain animals to acetonitrile. (3) Diet has a marked effect upon the resistance of animals to certain poisons; the resistance of some animals may be increased forty-fold by changes in diet. (4) Certain diets, notably dextrose, oatmeal, liver and kidney, greatly increase the resistance of mice to acetonitrile; their effect is similar in this respect to the administration of thyroid. (5) The effect of an oatmeal diet in increasing the resistance of certain animals to acetonitrile is probably due in part to a specific effect of the diet upon the thyroid gland; this is an illustration of how an internal secretion may be modified in a definite manner by diet. (7) Certain diets (notably eggs, milk, cheese, and various fats) greatly lower the resistance of certain animals to acetonitrile; their effect is the opposite of that of thyroid. (10) Diet causes distinct but not very marked differences in the resistance to morphine ". Graham (1915) states that " the feeding of carbohydrates to adult animals lessens their susceptibility to the production of liver necrosis by chloroform ". He also concludes that " the relative difficulty with which the characteristic central lobular liver necrosis can be produced in young pups after chloroform administration is in some way referable to the high glycogen contents of their livers ". Opie and Alford (1915) working on white rats found that a diet rich in carbohydrates protects the parenchymatous cells of the liver and of the kidneys from necrosis caused by chloroform, phosphorus, potassium chromate and uranium nitrate. Fat and meat

diets do not protect to the same extent as a carbohydrate diet. Opie and Alford refer to experiments conducted by Foster, who found that a protein poor diet inclines to protect dogs against ricin poisoning. Eisner (1931) found that rabbits, which when poisoned with small amounts of uranyl nitrate on an oats-water diet almost invariably survived when the diet contained beetroot, fresh green feed and the stems of cauliflower. Similar results were obtained both with the fresh and the boiled juice of cauliflower stems. Scholl (1932) increased the resistance of rats to phosphorus poisoning by feeding them on meal or dahlia bulbs. On discontinuing the feeding of meal the resistance decreased. On the other hand, a meal and dahlia diet had no effect on the susceptibility of rats to sodium cacodulate, atoxyl, and neosalvarsan. Couch (1932) states that lucerne hay, linseed cake and glucose retard the production of prussic acid in the gastro-intestinal tract, and also that cattle on a corn ration (starchy feeds) are more resistant to prussic acid poisoning. Cutler (1932) found that (1) a meat diet rendered dogs more susceptible to carbon tetrachloride poisoning as it tends to increase the production of guanidine, and (2) a calcium-rich diet and a carbohydrate diet is a preventive against carbon tetrachloride as the former diet antagonises guanidine and the latter relieves the hypoglycaemia. A calcium-rich diet to a certain extent is a protection against poisoning with oxalic acid and oxalates as these poisons cause the formation of insoluble calcium oxalate and decrease the diffusible calcium content of the blood. A diet rich in calcium also has a protective action in lead poisoning as calcium facilitates the fixation of lead in the tissues. Hence blood rich in calcium renders the system less susceptible to acute lead poisoning. Acid feeds facilitate the liberation of prussic acid from cyanogenetic glucosides, whilst alkaline foodstuffs have the opposite effect. Poisoning with iodides are counteracted by starchy feeds. The water content of the diet naturally plays an important rôle in the rate of dissolution and absorption of poisons. As a rule diets with a high carbohydrate content have a protective action against poisons as the liver is freely supplied with carbohydrates Hoekstra (1931) states that saponin not only increases the toxicity, but also favours the cumulative effects of the digitalis glucosides. Feeds which have an irritating effect on the gastro-intestinal mucosa, will be inclined to facilitate the absorption of poisons, hence increasing their toxicity. This effect is exerted by feeds with a high saponin content. Ewart (1931) states that the presence of small amounts of saponins facilitate the absorption of poisons as they increase the permeability of the epithelial layer of the alimentary canal without causing any injury to it. Plants containing tannin precipitate saponins to a certain extent, and hence protect against the effects of the latter. Plants rich in chlorophyl have a protective action against saponin poisoning as chlorophyl reduces the saponifying and haemolytic powers of saponins.

(*n*) *Tolerance and immunity in relation to plant poisoning.*—Tolerance and immunity must be distinguished from each other as they are used to describe two completely different phenomena as far as desensitization to plant poisons is concerned. Tolerance means an increase in resistance to plant poisons of a non-albuminoid nature, whilst immunity is not due to an habituation of the tissues to a

poison but to the development of specific antitoxins in the serum against plants whose active principles are of an albuminoid nature, for example, *Ricinus communis* L., *Adenia digitata* (Harv.) Harms. We must distinguish between a natural tolerance and an acquired tolerance.

(1) *Natural tolerance.*—With regard to natural tolerance we have to distinguish between the tolerance of the individual and the tolerance of the species. It is a well-known fact that animals belonging to the same class and even the same animal at different times, may vary considerably in susceptibility to poisons. On the other hand, there is a striking difference in the tolerance of different species of animals to poisons. Natural tolerance may be due to (i) diet, (ii) conditions which impede dissolution and absorption, or which effect changes in poisons present in the gastro-intestinal tract, (iii) the tissue cells being more active in the destruction (oxidation, reduction, decomposition), fixation, modification and elimination of poisons, and (iv) slow absorption from the point of application. These points have already been referred to.

(2) *Acquired tolerance.*—It was found (Steyn, 1932) that it was possible to cause the development of tolerance to *Chrysocoma tenuifolia* Berg poisoning in goats by repeatedly drenching these animals with small amounts of this plant; it was therefore decided to ascertain whether such a tolerance will also be developed in poisoning with other plants. It is a well-known fact that an active and specific immunity can be produced against those plants containing toxalbumins as active principles. These toxalbumins are abrin (*Abrus precatorius* L.), modeccin (*Adenia digitata* Engl.), crotin (*Croton tiglium* L.), crucin (*Jatropha curcas* L.), ricin (*Ricinus communis* L.), and robin (*Robinia pseudacacia* L.). This immunity, which must be distinguished from tolerance, may be developed to such an extent that an animal repeatedly treated with non-toxic amounts of the above toxalbumins may tolerate without any apparent ill-effects up to eight hundred times the minimum lethal dose. Jacoby (1924) states that according to Ehrlich (Zeitschr. f. Hyg. u. Infektionkrankh. 12 Bd. 1892) experimental abrin-immunity of mice is transmissible to the young. This transmission of immunity to abrin occurs through the milk, as the young of immunized mice show no immunity to this poison when suckled by mice susceptible to abrin, and the young of susceptible mice become immune when suckled by immune mice. Much progress has lately been made in the immunization of human beings against hay fever caused by the pollen of plants (hay fever), a problem to which many references will be found in the literature. Schamberg (1919) produced a tolerance to *Rhus toxicodendron* L. in human beings by giving *per os* small and increasing doses of the tincture to susceptible persons. Strickler, Schamberg's assistant, succeeded in preventing attacks of dermatitis in human beings caused by this plant by injecting them subcutaneously with an alcoholic extract of the plant. Schamberg has found that the " immunity " set up by his method generally does not persist longer than one month after the discontinuation of the administration of the tincture.

Sutton (1919) discusses the relation between anaphylaxis and immunity and, quoting Cooke, says that when few antibodies or none are present, the non-sensitive state exists, whereas when antibodies are numerous and attached to the body cells, the sensitive or anaphylactic state prevails; and when antibodies are in excess, with many unattached to body cells the immune state prevails. He states that " anaphylaxis and immunity are the same in principle differing only quantitatively ".

Ratner and Gruehl (1927-1928) demonstrated that normal guinea-pigs when exposed to an organic dust (horse dander) could become sensitised through inhalation. Guinea-pigs thus sensitised and subsequently exposed to the same dust after a suitable incubation period, exhibited unmistakable signs of anaphylaxis. Further experiments proved that typical respiratory anaphylaxis (bronchial asthma) can be produced in guinea-pigs by allowing them to inhale castor bean dust and again exposing them to this dust after an incubation period of two or three weeks.

Figley and Elrod (1928) refer to the occurrence of a large number of cases of asthma caused by the inhalation of castor bean dust liberated in the air from the pipes of a castor oil factory.

Petri (1930) mentions that a condition known as " fabismus " arises when the fruit of " *Vicia faba* " is eaten or when its pollen is inhaled. This condition, which is characterised by a rapid development of anaemia, icterus with urobilinuria, and swelling of the spleen and liver is supposed to be an " intolerance " to *Vicia faba*. Petri expressed no definite opinion as to whether this condition is due to direct poisoning or is an anaphylactic phenomenon.

Bürgi (1931) states that a tolerance to *Taxus baccata* L. (Yew) can be produced in horses by feeding them small amounts of the plant, and according to Kobert (1902) pigs, sheep and rodents acquire a tolerance to *Agrostemma Githago* L. after eating non-toxic amounts over certain periods.

Mackay (1931) was able to produce a tolerance to morphine in rats by administering this drug per os and found an increase of 70 per cent. in the weight of the adrenal glands in such morphine treated rats. Most of this increase had occurred in the cortex of the adrenals.

Tatum and Seevers (1931) made a valuable contribution to the study of drug addiction. They define addiction, tolerance and habituation as follows: " Addiction is a condition developed through the effects of repeated actions of a drug such that its use becomes necessary and cessation of its action causes mental or physical disturbances ".

" Tolerance is a condition developed by certain drugs such that progressively larger and larger quantities are required to produce the effects desired."

" Habituation is a condition in which the habitue desires a drug but suffers no ill-effects on its discontinuance."

Some drugs produce addiction and no tolerance (cocaine) and *vice versa* (organic nitrites) while others produce both (morphine).

With regard to strychnine and cocaine Tatum and Seevers state that experiments on animals point to increased sensitivity rather than tolerance. They state furthermore that tolerance appears to be developed only to that class of drugs which produce a reduction in the activity of cells and increased sensitivity to those drugs which increase the activity of cells.

Biggam, Arara and Ragab (1932) refer to drug-addiction in Egypt in which heroin, opium, morphine, hashish, manzoul, cocaine and mixtures of these drugs are concerned. The withdrawal symptoms exhibited by these addicts are restlessness, sleeplessness, excitability, irritability, sneezing, yawning, lachrymation, colic, diarrhœa, headaches, vomiting, and pains in the limbs. These symptoms persist for about four days and then subside. They have found that a substitution therapy with atrophine, morphine, strychnine, paraldehyde, luminal and magnesium sulphate relieve the withdrawal symptoms very markedly.

Santesson (1932) succeeded in producing a tolerance in rabbits to copper sulphate by injecting them subcutaneously with small and increasing quantities of this salt.

Simpson and Banerjee (1932) state that horses develop a tolerance to *Abrus precatorius* L. when the seeds are given in small and gradually increasing doses.

Speight (1932) states that ill-health and insanity are inevitable results of the excessive and continued use of dagga (*Cannabis sativa* Lam.).

It is a most interesting phenomenon that a tolerance is developed to one poison whilst another will cause a hypersensitivity when taken in repeated small amounts. Of still greater interest is the fact that a certain organ may develop a tolerance to a certain poison, whereas another organ will become sensitised to the same poison. As an example of the latter type of poison Caffeine, which causes desensitization of the kidney and sensitization of the central nervous system, may be quoted.

Animals, which have developed a tolerance to some or other poison (acquired tolerance) can hardly be regarded as normal as some or other physiological or (and) histological change is bound to have occurred in their system. In experiments at Onderstepoort sheep developed tolerance to *Centaurea picris* D.C. whilst they became more sensitive to *Asclepias physocarpa* Schltr. (Steyn, 1932). Rabbits after having received a preliminary treatment with increasing doses of potassium cyanide appear to develop a tolerance to this poison (Steyn, 1932b).

The nature of acquired tolerance is still an unsettled problem. This acquired state of desensitization is probably due to a mobilization of the defensive powers of the system and the following suggestions are advanced by the author with regard to the development of acquired tolerance to poisons:—

(i) *Cellular tolerance.*—When living cells are brought into contact with low but increasing concentrations of poisons, it is possible that these cells will in the course of time adapt themselves to their new environment and perform their functions in a normal way in spite of the fact that they are bathed in a fluid which under normal circumstances would have seriously interfered with their activities. To further elucidate this point I might mention drug fast bacteria and protozoa. This cellular immunity may be intracellular or extracellular or both. That is, the cells may allow the poison to enter into their interior and they may in some or other way inactivate or destroy the poison within their structure; or, they may develop their defensive powers to such an extent as not to allow the poison to enter into their protoplasm. The latter process may be termed "selective osmosis", and is probably responsible to a large extent for decreased absorption.

(ii) *The production of antibodies* (*immunity*). No proof has as yet been brought forward to support the hypothesis that acquired tolerance to non-albuminoid poisons is due to the production of antibodies.

(iii) *Increased rate of inactivation, elimination and destruction of the poison.*—It would appear possible that the activities of the body tissues and the liver, as detoxicators, and of the organs of excretion (liver, kidneys, skin, gastro-intestinal mucosa, lungs, lactating glands) may be increased when sufficient time is available in order to allow of the development of such an increase in activity by gradually introducing into the system non-toxic and increasing amounts of a poison. Mackay (1931) found that the weight of the adrenals of rats increased by about 70 per cent. when these animals receive gradually increasing doses of morphine sulphate. This may be an attempt of the body to combat the effects of the poison. Tolerance to alcohol and increased susceptibility to hydrochinon are due to an increase in the oxidising capacity of the system which is brought about by repeated administrations of these substances (Vollmer, 1932 a). The oxidation products of alcohol are relatively non-toxic, whilst those of hydrochinon are more toxic than hydrochinon itself.

(iv) *Decreased rate of absorption of the poison.*—It is clear that the efficiency of the organs of absorption and elimination will to a large extent determine the susceptibility of such an animal to poisons.

(*o*) *Idiosyncrasy* (*hypersusceptibility*).—Again we must distinguish between natural and acquired idiosyncrasy to poisons. The author considers that natural idiosyncrasy may be due to (1) the organ already weakened by disease is attacked by a certain poison, (2) decreased rate of inactivation, elimination and destruction of the poison by the tissues, (3) increased rate of absorption of the poison, and (4) certain tissues being extremely sensitive to the action of some poisons. On the other hand, acquired idiosyncrasy may be due to (1) sensitization due to ingestion of small amounts of plants containing toxalbumins, (2) the cumulative action of poisons, and (3) to the fact that the harmful effects of a substance is increased by another poison being already present in the body.

The above points have already been referred to in previous discussions. With regard to idiosyncrasy being due to the synergistic effects of poisons, metaldehyde and menthol could be mentioned. Leibbrandt and Mayer (1929) found that these two substances, when administered at the same time, increased in their toxicity to human beings and rabbits. It is furthermore a well-known fact that drugs with synergistic effects, when administered at the same time or within short periods of each other, increase each others actions to a degree much higher than the sum of the actions of the individual drugs.

(*p*) *Direction of passage of poisons through organs.*—Kahlson (1932) drew attention to the fact that the action of a drug depends on the direction in which it passes through a cell-group. Substances of the muscarine group are much more active when passed into the heart by means of a canula, than when the whole heart with sinuses and atria is submerged in solutions of these poisons. It was established that in both cases the heart contained equal amounts of the poison. Acetylcholin causes a much more marked increase in peristalsis when it passes through the intestinal wall in the direction mucosa-serosa, than when it passes in the opposite direction (serosa-mucosa).

(*q*) *Other conditions, which may bring about a change in susceptibility to poisons.*—Vollmer (1930) found that preliminary treatment with caseosan increased the resistance of white mice to alcohol. This phenomenon is ascribed to an increase in the oxidation processes caused by the caseosan. This increased tolerance lasts from four to five days. Treatment of white mice with caseosan had no effect on their susceptibility to morphine, colchicine and hydrochinon. Similar results were obtained, in white mice treated with caseosan and subsequently subjected to the effects of sufrogel. Vollmer and Buchholz (1930) reduced the susceptibility of white mice to alcohol by preliminary treatment with thyrocin, sodium lactate, glucose and methylene blue. This treatment, however, increased susceptibility to colchicin and hydrochinon. Preliminary treatment with thyroxin caused no change in susceptibility to morphine. The changes in susceptibility to the above drugs are held by Vollmer and Buchholz to be due to an activation of the oxidation processes in the body. Vollmer and Behr (1930) confirmed the results of experiments conducted by Riesser and Hadrossek in that they found that the irradiation of white mice with the Osram-Vitalux lamp (white glass) increases the tolerance of these animals to alcohol. One irradiation has the same protecting effect against alcohol as repeated irradiations. This protecting effect is present only half to one and a half hours after the last irradiation. Similar results were obtained by irradiations with the Heraeus-Mercury-Quartz lamp. Irradiation with these two lamps increased the susceptibility of white mice to hydrochinon and colchicin and had no effect on their susceptibility to morphine. These changes in susceptibility brought about by irradiation are attributed to an increase in the oxidation processes in the body. Vollmer (1931 a) experimenting upon white mice and rates found that (*a*) a single injection of glucose does not affect the narcotic action of ethylalcohol; (*b*) a too intensive irradiation of the

animal especially with the Heraeus-Quartz lamp, does not detoxicate ethylalcohol and aniline or increase the toxicity of hydrochinon. This effect is due most probably to an inhibition of the process of oxidation; (*c*) preliminary treatment with thyrocin, glucose, methylene blue, or sodium lactate increases the action of anilin; irradiation of the animal or preliminary treatment with caseosan has no effect on the action of anilin; (*d*) the actions of strychnine could in no way be influenced; (*e*) animals subjected to a preliminary treatment with caseosan or glucose showed a slightly increased susceptibility towards salvarsan; and (*f*) preliminary treatment of the animals with thyroxin, glucose, caseosan or irradiation with the Osram-Vitalux or the Heraeus-Quartz lamp had no effect on the action of methylalcohol, iso-propylalcohol, n-propylalcohol, iso-butylalcohol and n-butylalcohol.

Not only mature tissues but also embryonic cells are capable of developing a tolerance to poisons. Wilson (1922) draws the following conclusions from his experiments: " Embryonic mesemchyme cells, cultivated in weak solutions of copper sulphate and sodium arsenite, develop in the course of two days on acquired intracellular tolerance for strong doses of these two poisons ".

XII. CONDITIONS GIVING RISE TO ACCIDENTAL INGESTION OF POISONOUS PLANTS.

The following conditions are considered by the author to give rise to accidental plant poisoning in stock:—

A. *Under drought conditions animals, in the absence of edible vegetation, or when such vegetation is scanty, are forced to feed on poisonous plants.* As an outstanding example *Geigeria passerinoides* Harv. may be mentioned. Veld burning should also be mentioned as it frequently is the cause of plant poisoning, when stock are allowed to graze on recently burnt veld. On such grazing deeprooted poisonous plants (*Dichapetalum cymosum* Hook, *Pachystigma pygmaea* Robyns) and bulbous plants (*Urginea Burkei* Baker) sprout more quickly than grass and hence constitute a great temptation and danger to stock.

B. *In winter and in early spring before the summer rains have fallen* when there is no or hardly any edible green herbage, animals are attracted by the green leaves and flowers of poisonous non-deciduous trees and shrubs (*Acokanthera venenata* G. Don) and by other poisonous plants, which having deep roots or bulbs are not dependent upon spring rains for the production of leaves and flowers (*Dichapetalum cymosum* Hook, *Urginea Burkei* Baker). Allowing stock to graze on old mealie lands in winter and early spring, a general practice in South Africa, should also be mentioned here. The edible vegetation on such lands is mostly dry with the result that the animals are very much tempted to eat poisonous plants which are fresh and green [*Dimorphotheca spp.* (bietou)] or succulent (*Cucumis myriocarpus* Naud). It is obvious that thirsty animals will eat any green plants appearing in an otherwise dry veld and in this way poisonous plants become particularly dangerous.

C. It frequently happens that through the intergrowth of normal veld vegetation and poisonous plants, animals are unable to avoid the ingestion of poisonous plants when these grow in close association with grass. Striking examples of this intergrowth can be seen in grass veld infested with *Moraca* and *Homeria* spp. (tulips) and *Geigeria* spp. (specially *Geigeria aspera* Harv.).

D. Plant poisoning is of very frequent occurrence in transport and draught animals, as these hungry animals, when outspanned, partake greedily of practically any plant. In earlier days "transport riders" suffered severe losses among their oxen as they usually outspanned in valleys (vleis) near water where there frequently was abundant growth of *Homeria*, *Moraea* spp. (tulips) and *Dimorphotheca* spp. (bietou).

E. Animals imported from overseas or animals introduced into new areas often fall easy victims to poisonous plants, not being familiar with the vegetation they are unable to exercise their sense of discrimination. Acclimatization, therefore, not only implies the immunization of animals, against infectious diseases, but also a development of a sense of discrimination between edible and poisonous plants. Stable-fed animals and animals brought up under unnatural conditions also lose this sense of discrimination. Again we have to consider the possibility and probability of animals which have been reared in areas infested with poisonous plants, having developed a tolerance to these plants after the ingestion of non-toxic amounts over prolonged periods.

F. Poisonous plants may be accidentally ingested with hay, and in this way *Moraea spp. Homeria spp.* (tulips), *Ornithogalum thyrsoides* Jacq., and *Crotalaria dura* Wood and Evans, have caused heavy losses.

G. Aphosphorosis causes a craving for substances which animals in normal circumstances would not ingest. This condition may prompt animals to partake of poisonous plants.

H. Animals may acquire a craving for certain poisonous plants and other animals may follow their example. Animals are said to develop a craving for the loco weed (*Astragalus sp.*) in Western United States and for *Swainsonia spp.*, "*Cucumis myriocarpus*" and other plants (Seddon, 1930). This phenomenon may to a certain extent be responsible for sudden outbreaks of plant poisoning. In this respect sudden changes in the toxicity of plants, a fact which has been well established, must also be considered.

I. Certain plants may need certain constituents of other plants in order to liberate their active principles. This may be the case with plants containing cyanogenetic glucosides, from which prussic acid can be liberated by the action of a ferment contained in another plant or plants. In Australia it was found that the cyanogenetic glucoside contained in *Eremophila maculata* Fv. M. required an enzyme present in *Acacia Georgina* Bailey for its decomposition into prussic acid (Finnemore and Cox, 1927).

J. The evil of overstocking, which is practised in South Africa to an alarming extent, is responsible for the death of thousands of animals from plant poisoning yearly. The veld is denuded of valuable edible vegetation and the stock are left the choice of either dying from starvation or from poisonous plants. Overstocking in many cases is the cause of the repid spread of poisonous plants as they are left to grow and seed, whilst the valuable edible plants are continuously eaten and may eventually completely disappear. A fact worthy of mentioning is that plants which constitute the sole diet of animals may cause severe losses but when eaten together with other vegetation they may be harmless or even valuable in times of drought. This is the case with *Geigeria passerinoides* Harv. and *Chrysocoma tenuifolia* Berg. (Steyn 1932 a).

XIII. PREVENTION OF PLANT POISONING.

The author considers that losses from poisonous plants can be combated very successfully by (*a*) keeping animals away from dangerous areas; (*b*) allowing stock access to dangerous areas when the plants concerned are least toxic; (*c*) exercising special care during drought periods; (*d*) paying special attention to stable-fed animals, *trek* animals and stock newly introduced into areas where poisonous plants occur; (*e*) fighting the evil of overstocking; (*f*) allowing animals access to water before they are allowed to graze on reaped lands where the edible herbage is dry and on which green and succulent poisonous plants occur; (*g*) allotting grazing on which poisonous plants occur to those classes of stock which are less susceptible to the effects of the plants concerned; (*h*) using the rotation camp system; and (*i*) preventive treatment.

A. Keeping Animals away from Dangerous Areas.

This seems so self-evident that it would be a waste of time to remark on it. In areas where poisonous plants abound those portions of the farms infested with such plants could be used, if suitable, for the cultivation of crops. This has, for example, been successfully done on farms where *Dichapetalum cymosum* Hook (gifblaar) and *Melianthus comosus* Vahl abounded.

B. Allowing Stock Access to Pastures containing Poisonous Plants, but at a Time when they ar not Toxic or only slightly so.

Many plants are most poisonous in the pre-flowering and flowering stages, hence grazing where such plants occur should be used when these plants are in their last stages of development. The fact that deeprooted and bulbous poisonous plants sprout before the spring or summer rains, which are essential for the growth of grass and many other kinds of edible herbage, should also be considered here. It is obvious that animals will be tempted by any green vegetation when the rest of the herbage is dry. It is for this reason that in spring and early summer such severe losses among stock are sustained from *Dichapetalum cymosum* Hook and *Urginea burkei* Baker poisoning. In addition, the young leaves of the former plant are much more toxic than the mature ones. Veld abounding

with *Dichapetalum cymosum* Hook could, therefore, be used at very little risk when this plant has very few young leaves or mature leaves only (see *Dichapetalum cymosum* Hook). At times when prussic acid poisoning is likely to occur through the ingestion of wilted grasses, it would be of some value to allow animals to graze in the early morning as the hot afternoon sun will cause an increased production of prussic acid in the grasses concerned.

C. EXERCISING SPECIAL CARE DURING PERIODS OF DROUGHT.

It is obvious that during periods of drought and scarcity of grazing animals will be more likely to partake of poisonous plants. In many cases they are left with the choice of dying from starvation or the effects of poisonous plants. Valleys (vleis), although they may constitute valuable grazing in times of drought, may on the other hand be dangerous owing to the prevalence of poisonous plants.

D. PAYING SPECIAL ATTENTION TO STABLE-FED ANIMALS, TREK ANIMALS AND STOCK NEWLY INTRODUCED INTO AREAS WHERE POISONOUS PLANTS ABOUND.

Stable-fed, trek and transport animals and animals newly introduced into areas are, when turned out to graze, much more likely to ingest poisonous plants than animals accustomed to the areas concerned and running under natural conditions owing to a decreased sense of discrimination between edible and poisonous plants. This decreased sense of discrimination in these animals is due to artificial conditions, hunger and unfamiliarity with new environments.

E. FIGHTING THE EVIL OF OVERSTOCKING.

It is not intended here to go into details as this point is elucidated under " Conditions giving rise to the accidental ingestion of poisonous plants ".

F. ALLOWING ANIMALS ACCESS TO WATER BEFORE THEY ARE ALLOWED TO GRAZE ON REAPED LANDS WHERE THE EDIBLE HERBAGE IS DRY AND ON WHICH GREEN AND SUCCULENT POISONOUS PLANTE OCCUR.

The grazing of harvested lands, especially mealie lands, is practised very extensively in South Africa. In many cases most of the edible vegetation on such lands is dry hence any harmful and poisonous plants which are green (tulp) or succulent (*Cucumis myriocarpus* Naud.) will constitute a great temptation to animals grazing on such lands. This temptation will be greater when these animals are thirsty, hence the necessity of allowing the animals access to water before they are allowed on to the lands.

G. ALLOTTING GRAZING ON WHICH POISONOUS PLANTS OCCUR TO THOSE CLASSES OF STOCK WHICH ARE LESS SUSCEPTIBLE TO THE EFFECTS OF THE PLANTS CONCERNED.

The fact that some classes of stock when grazing on areas where poisonous plants occur, do not develop symptoms of poisoning may be due to the animals not eating the plant or plants, or to their being persistant to the poison contained in the plant or plants

concerned. On farms where *Matricaria nigellaefolia* D.C. poisoning occurs in cattle, the grazing could be utilized for sheep and horses as these animals were found by Andrews (1923) to be resistant to the poison.

H. Using the Rotation Camp System.

The removal of poisoned animals from one camp to another often decreases the morbidity and mortality although the poisonous plants which are the cause of the trouble are also present in the new camp. The reason for the decrease in mortality is probably due to the fact that the animals being in a new environment eat plants other than the poisonous ones for the first few days at least. The rotation camp system is of the greatest value in areas overgrown with plants which are poisonous in large amounts only (*Geigeria passerinoides* Harv.).

I. Preventive Treatment.

As a rule preventive treatment in plant poisoning is of little value. Great success has, however, been achieved in some cases by (*a*) additional feeding, and (*b*) preventive treatment with drugs.

(*a*) *Additional Feeding.*—This can be done only on farms with an adequate supply of water for the growing of crops or by those farmers who can afford to buy the necessary foodstuffs. It stands to reason that animals when allowed some substantial feed in the morning before being turned out to graze, will exercise more discrimination in feeding than when turned out hungry. In addition, some plants (*Geigeria* spp., *Chrysocoma tenuifolia* Berg) when eaten alone, will cause poisoning whilst when taken together with edible plants will not be harmful or may even be of good feeding value.

(*b*) *Preventive Treatment with Drugs.*—Great success has attended the preventive treatment of poisoning with plants containing prussic acid or cyanogenetic glucosides by means of sulphur. As a rule the addition of 5 to 10 per cent. of sulphur to licks suffices to prevent losses, but at times when conditions are favourable for heavy outbreaks of prussic acid poisoning (see *Gramineae*), it is recommended to dose the animals with sulphur in addition to allowing them access to licks containing sulphur.

In areas where there is danger of oxalic acid or oxalate poisoning (*Mesembrianthemum* spp., *Psilocaulon* spp.) it would certainly be of value to supply stock with licks containing slaked lime as it will to a certain extent form the practically insoluble calcium oxalate, which will be passed out with the faeces.

XIV. ERADICATION OF POISONOUS PLANTS.

The adage "prevention is better than cure" is very appropriately applicable to the problem of plant poisoning. Once animals have developed symptoms of poisoning very little can be achieved with treatment, as the poison has already found its way into the blood circulation. In addition it frequently happens that when symptoms are noticeable irreparable harm has been done in some or other vital organ. This is almost invariably the case in poisoning with *Pachystigma pygmaea* Robyns and *Senecio* spp. The

sooner it is realised that with a few exceptions the treatment of large herds or flocks of stock for plant poisoning is useless, the better it will be for stock breeding in South Africa. In cases where the veld is overgrown with poisonous plants (*Tribulus terristris*, *Geigeria passerinoides*) on which the stock are dependent to a large extent for their food supply, treatment is of no value at all, as animals will, as soon as they are released after treatment, again ingest such plants. This is the case in areas where severe outbreaks of disease caused by *Geigeria passerinoides* Harv. occur. It is for the above reasons that every attempt to eradicate poisonous plants should be made. It is realised that it is almost an impossibility to eradicate those plants, which do not grow in patches and whose underground portions penetrate the soil very deeply. This is the case with some species of *Homeria* and *Moraea* (tulips), which occur so extensively in valleys and whose corms are deeply rooted, and the eradication of which will entail an enormous amount of labour and capital. Many poisonous plants can, however, be eradicated at a comparatively low cost. The methods of eradication depend on the nature of the growth and propagation of the plants concerned. Annuals produce flowers and seeds in one season and can be effectively eradicated by hand pulling or digging up. Biennials take two years to produce seeds and they may be eradicated in the same way as the annuals; they will also succumb to continued cutting down. Perennials are propagated both by seed and underground rootstocks, bulbs, corms and tubers. They may be dug up when not too deeply rooted, or sprayed. The following methods may be used in attempts to eradicate poisonous plants.

A. Spraying.

In discussing weed killers the most important point that arises is the possibility of their causing poisoning in stock. It is obvious that weed killers which are to be extensively used on pastures should not be so toxic as to cause poisoning in such amounts as are likely to be ingested with the vegetation. The toxicity of arsenical compounds precludes their use as weed killers except in special cases, for example, in localised spots.

Another point of the utmost importance in the extensive application of weed killers to pastures is the degree of damage they will cause to the edible and valuable vegetation in such solutions as will destroy the weeds. This relative destructive value of weed killers both to weeds and edible vegetation is perhaps of more importance than the degree of toxicity of the weed killers to stock, as the poisoning of stock could be prevented by not allowing them access to treated pastures until after heavy rains have fallen.

From the results of experiments conducted by Fröhner (1919), Seddon and McGrath (1930) and by the writer at Onderstepoort, it would appear that sodium chlorate is relatively speaking, not very toxic to stock. As reports from New Zealand [Editorial 1930 (a) and Lyons, 1930] and the United States of America (Editorial 1931) record it to be an efficient weed killer, sodium chlorate would best seem to satisfy the requirements for a suitable weed killer to be utilised on pastures.

However, before its use as a general weed killer on pastures can be advocated, it is essential to conduct experiments in order to determine its relative destructive capacity for the weed or weeds to be killed and for the pasture plants. It is on this property that the suitability of sodium chlorate as a weed killer on pastures depends.

Leaves with uneven, rough and hairy surfaces retain more spraying material than leaves with smooth and hairless surfaces, hence weed killers will have a more pronounced effect on the former leaves. Plants should be sprayed on a clear day and when in their early stages of development, as in these stages they are less resistant and also less spraying material is needed. The following weed killers may be found useful: (*a*) Common salt. It is used in a 20-30 per cent. solution, and is a cheap and safe spray. It is very destructive when applied in hot and dry weather as it kills plants by absorbing moisture. (*b*) Caustic soda. Used as a spray in 5 per cent. solution it will kill practically all vegetation until washed out of the soil by rain. (*c*) Blue stone (Copper sulphate). It can be used in a 2-3 per cent. solution. (*d*) Sodium chlorate and calcium chlorate. Sodium chlorate is the cheaper of the two salts and is also more effective. It is very effective for destroying thistles and many graminaceous weeds. It is recommended in 2·5-10 per cent. solutions. (*e*) Arsenical preparations: on account of their toxicity these preparations can only be used in localised spots to which stock should have no access until after heavy rains have fallen. The eradication of *Dichapetalum cymosum* Hook (gifblaar) with arsenical preparations proved a failure. Investigations into methods of eradicating the plant are being continued by the Division of Plant Industry, Pretoria.

Favourable reports were received from farmers who have attempted the eradication of *Pachystigma pygmaea* Robyns by means of spraying it with locust poison. (*f*) Copperas (iron sulphate). It is recommended in a 25 per cent. solution.

Before applying the above weed killers to extensive areas of grazing it would be advisable to conduct preliminary experiments on a small scale in order to determine an effective strength of the weed killer and also to what extent it will destroy valuable pasture plants.

B. Crowding Out of Edible Plants.

Overstocking and veld burning are two factors which greatly assist in the spread of poisonous plants and weeds. Areas abounding with poisonous plants may be reseeded with desirable grasses or other edible vegetation (salt bush). As many poisonous plants are more resistant than edible herbage it is doubtful whether this method of eradicating undesirable plants will be of much value.

C. Close Grazing.

If we accept as true that a herd or flock of animals grazing over the same area ingest approximately the same plants we can conclude that the more animals there are to a unit of area the less of the poisonous plant or plants present in such an area will each animal ingest. The less toxic the plant or plants are the smaller will be

the risk of the animals becoming poisoned. Many farmers temporarily overstock camps infested with *Pachystigma pygmaea* Robyns without the animals developing *gousiekte*. It stands to reason that the abundance of poisonous plants on the areas concerned is of the greatest importance in the determination of the degree of safety of such areas as grazing.

D. Digging Up.

The most effective method of eradicating plants is to uproot them, but unfortunately this method is for various reasons not always practicable. By this method many farms have been rid of *Urginea burkei* Baker, *Urginea macrocentra* Baker, and various species of *Cotyledon*. The most economical method of keeping farms free from weeds and poisonous plants is to hire somebody especially for the purpose of digging up such plants. It is astonishing the size of the area which can be cleared of undesirable plants by one man in a month's time.

E. Veld Burning.

Some farmers maintain that persistant burning of veld where *Pachystigma pygmaea* Robyns occurs renders this plant non-toxic. Even if this statement were true it would seem advisable to attempt the eradication of this plant rather than practice veld burning with all its attendant detrimental effects on the grazing.

The eradication of poisonous plants, especially annuals from cultivated lands is a comparatively easy task and is practised by many farmers by allowing lands to lie fallow and then ploughing them again before the weeds reach the seeding stage.

XV. LEGAL ASPECT OF PLANT POISONING.

Owners of property may be held responsible for losses among stock due to poisonous plants growing over or through fences, or when cuttings from such plants are disposed of in such a way as to be accessible to stock. Also sellers of foodstuffs containing poisonous plants may be called upon to pay the damage done among animals by such plants.

With regard to the presence of portions of poisonous plants in wheat (bread poisoning) the following regulation [12 (7)] of the Food, Drugs and Disinfectants Act (No. 13 of 1929) has been enacted: " Every mill in which grain is milled for human consumption shall be provided with efficient sieving and winnowing appliances so as completely to remove the seeds of Senecio (Springkaanbos) and every other poisonous or unwholesome seeds or matter. Any person selling any flour or meal containing such seeds or matter shall be guilty of an offence ".

XVI. INVESTIGATION OF PLANT POISONING IN THE FIELD.

In order to conduct a satisfactory investigation of outbreaks of plant poisoning, it is not only necessary to possess a good knowledge of the symptomatology, pathology and botanical aspect of plant poisoning, but it is also essential to have a good knowledge of

psychology in general and in particular of those people with whom one has to deal in the course of such investigations. A reliable diagnosis is often to a very large extent dependent upon the information supplied by the stock-owners concerned or by the managers of their property. It frequently happens that by the time an officer arrives at a farm where cases of plant poisoning occur the owner has already made a diagnosis and supplies information which gives support to his diagnosis and does not state facts as they are. This misleading information, which is not always given intentionally, sometimes makes a definite diagnosis impossible. For example, in different camps on a farm different poisonous plants, which causes similar symptoms in stock, may abound and if the stock owner does not supply the true history of the case, it would be a difficult task to trace the plant that caused the trouble unless definite information is obtained from the examination of the gastro-intestinal contents.

Another problem which is encountered among land and stock owners is their reluctance to acknowledge the presence of poisonous plants on their farms. Most farmers want to protect the "good names" of their property and simply will not believe that there are poisonous plants on their farms. It is obvious that the information supplied by such farmers to investigating officers is misleading in every respect. This matter is further complicated when cases of plant poisoning occur on farms that are in the market. Many farmers who know or suspect poisonous plants to occur on their property are desirous of disposing of such properties. It is "business" to advertise such farms as well as possible and for this reason no mention will be made about the prevalence of poisonous plants. Many farmers who have bought farms in areas with which they are not practically acquainted, have discovered to their cost that the farm they bought is badly infested with poisonous plants.

XVII. METHODS OF ASCERTAINING WHETHER PLANTS ARE TOXIC OR NOT.

The following method of procedure is adopted by the author in the investigation of suspected poisonous plants.

If a plant not known to be poisonous is suspected of having caused poisoning in stock, there is only one way of ascertaining whether the suspected plant is the cause of the trouble or not, and that is to cause a member or members of the class or classes of stock concerned to take such a plant or to force feed such animals with the plant in question. It is hardly necessary to state that the whole plant, or parts, of the plant concerned should be fed to or introduced into the experimental animals, as very unreliable results will be obtained by using the expressed juice of the plant or extracts prepared from it. Other points of the utmost importance in the investigation of the toxicity of plants are:—

A. The use of experimental animals which belong to the same class or classes of animals which are suspected of having been poisoned by the plant or plants concerned. This is obvious as there is a great difference in the susceptibility of the different classes of

animals to poisons. The fact that some plants may cause the development of tolerance in animals running on farms where such plants occur must be considered in the choice of experimental animals.

B. The use of the same parts of the plant, which must, if possible, also be in the same state and stage of development as the plant suspected of having caused the poisoning. The plants or extracts (if extracts of plants are concerned) must be administered in the same way in which they were taken by the victim.

C. The keeping of the experimental animals, if possible, under the same conditions as those animals in which the poisoning occurred (for example in plants causing photosensitization). It is unnecessary to go into details as the above points have already been fully referred to under "Factors concerned in the Determination of the Toxicity of Plants".

With regard to the investigation of suspected cases of poisoning in human beings it is a more difficult task to draw definite conclusions as we have to apply results obtained from experiments conducted upon animals to human beings. It goes without saying that the identical material (extracts, parts of the plant, etc.), suspected to have caused the poisoning in the human beings must, wherever possible, be used in the experiments to determine their toxicity, or when these are unobtainable, similar preparations should be made.

Once a plant is suspected of having caused poisoning in stock, the following methods of investigating the problem may be followed:

A. Grazing and Tethering Experiments.

This is the most natural way of ascertaining whether a plant is toxic or harmless. Unfortunately it is not always practicable, especially in a country like South Africa with such vast dimensions, it would entail too much time and expense to conduct such experiments in a reliable manner. It must, however, be realised that those cases of plant poisoning, in which soil and climatic conditions play a rôle, must be investigated on the spot where and at the time when they occur, as the forwarding of the suspected plants to a laboratory for investigation is of no value. This is the case especially with plants suspected of causing photosensitization (*geeldikkop* and *dikoor*) and also with plants which are poisonous when wilted (prussic acid). Patches where the suspected plant occurs must be cleared of all other vegetation and the experimental animal or animals confined to these patches by means of movable enclosures or by tethering. The disadvantages of this method are that the amount of plant taken in cannot be accurately controlled and that some animals may refuse to take the plant.

B. Feeding Experiments.

Although it is of great value that the experiments with suspected poisonous plants be conducted on the spot where such cases of poisoning have occurred it is for various reasons not practicable in most cases. The large majority of suspected plants are, therefore,

forwarded to the laboratories for investigation. Although the feeding of suspected plants to animals must be resorted to when such amounts as cannot be force-fed are required to produce poisoning, this method has disadvantages, which will be shortly discussed. In most cases of feeding experiments under laboratory conditions it is a most difficult task to induce the experimental animals, even after days of starvation, to take the plants offered to them, as they are accustomed to the ordinary routine foodstuffs. The animals may take small amounts of the plant fed and may develop a tolerance to that particular plant with the result that wrong conclusions are drawn: Again definite amounts of some poisonous plants must be eaten within a certain time in order that they may exert their toxic effects. Certain quantities of plants containing prussic acid may, for example, cause death when ingested within one hour, whilst equal amounts will have no effect when eaten within three hours. Feeding experiments do not allow of the accurate calculation of the amounts of plants eaten owing to wastage and, also loss in weight of fresh and green (succulent) plants due to loss of moisture. Very frequently suspected plants are mixed with foodstuffs and then presented to the experimental animals. Some plants are poisonous only when fed alone or in large amounts (*Geigeria passerinoides* Harv., *Chrysocoma tenuifolia* Berg). Furthermore, it may be essential that the suspected plant must be ingested very soon after collection as it may decrease in or lose its toxicity. This is the case with plants containing prussic acid or volatile active ingredients. Another important factor is that in feeding experiments the animals have to be isolated. As many as twenty-seven different plants have been received at Onderstepoort on one day. The amounts of space taken up by animals fed and the amount of time spent on weighing the material before and after feeding, can easily be appreciated.

C. Force Feeding Experiments.

Animals which refuse to take suspected plants may be forced to do so by balling with the hand or balling-gun or by drenching them. The disadvantages of this method are that the normal processes of mastication and deglutition are excluded. In addition in drenching plants a large amount of water has to be added to most of them in order to render the plant drenchable. The errors that may arise from this method are firstly that mastication may decrease the toxicity or prevent, to a certain extent, the liberation of the active principles of some plants. The large amount of alkaline saliva secreted by sheep, horses and cattle during the act of feeding will reduce the action of those plants, which exert their effects most markedly in an acid environment, and will also retard the liberation of prussic acid from cyanogenetic glucosides which are dependent upon an acid environment for their decomposition. Secondly, the water added to the plant material to be drenched may, and probably will in many cases, increase the rate of absorption of the active principle, thus rendering the toxic and lethal doses smaller than they actually will be when the plant is eaten. The author has attempted, time and again, to determine the relative toxicity of *Dichapetalum cymosum* Hook when fed alone, fed mixed with foodstuffs, balled alone, balled mixed with foodstuffs, and when drenched

by stomach tube. No definite results were achieved as the animals when fed refused to take the plants. It was, however, found that the plant was slightly more toxic when drenched than when balled, especially when water was added to the plant a long time before drenching. This result is quite conceivable as the active principle of this plant is water soluble.

Another disadvantage of the method of drenching is that a large amount of plant material is introduced into the stomach (rumen in ruminants) within a very short time. This naturally allows of and leaves little time for excretion of the active principles, quick absorption of the plants concerned, with the result that these plants will be more toxic when drenched than when eaten.

It has been stated by some critics that the drenching of plant material will cause some of the drenched material to flow right through the stomach (fore-stomachs in ruminants) into the small intestine, with the result that the undigested plant material will cause intestinal irritation with consequent diarrhœa. If the drenching is executed with any amount of discretion, this will not happen. Drenching experiments conducted by the author upon unstarved animals showed that amounts of plant material measuring up to five litres very rarely, and amounts up to three litres never found their way into the omasum and abomasum.

In addition large numbers of ruminants and non-ruminants are repeatedly drenched yearly at Onderstepoort with plants, and none of the experimental animals have developed diarrhœa after having been drenched with a non-poisonous plant.

Another disadvantage of the drenching method is that plants containing gaseous (prussic acid) or volatile active ingredients, lose a large percentage of these ingredients in the course of preparing the plants for drenching. Such plants when drenched will, therefore, be less toxic than when eaten.

The advantages of this method, however, are that (*a*) the plant finds its way into the animal very soon after collection; (*b*) the dosage is accurate; (*c*) a minimum of time for the completion of the experiments is required, and (*d*) all animals used in different experiments can be placed in one stable or enclosure and need not be isolated as in the case of feeding experiments.

The method of balling is only of value when small amounts of plant material are to be dosed, as otherwise it would entail an enormous amount of time especially if a number of plants are for investigation received at the same time.

If we consider the foregoing discussion, it appears that the method of drenching by stomach tube is the most reliable and most time-saving method, except in cases where animals have to take in large amounts of plant material daily, in which case it would be impossible to drench such amounts. It is for the above reasons that the method of drenching experimental animals by stomach tube has been adopted at Onderstepoort as a routine method, except in those cases where animals will take the plant material voluntarily without excessive starvation.

The following technique is employed by the author in the preparation of the plant material and drenching of the animals.

D. Preparation of the Plant Material for Drenching.

It is impossible to put on paper the many details in the methods of preparation of plant materials for drenching; these can be acquired through personal experience only.

As already stated, plants should be administered in the stage of development and state in which they are suspected of having caused poisoning and also by the route through which they have entered the system. Very serious mistakes have been made by expressing the juice of plants and drenching these to animals, or by preparing extracts of plants and drenching these, or even injecting them into animals. If reliable results are to be obtained the plant as such, and not its juice or extracts prepared from it, should be administered by stomach tube, unless of course the juice or extract is suspected of having caused poisoning. It is also essential that the prepared plant material should not be left mixed with water for a long time before drenching, as non-poisonous plants may in this way be rendered poisonous. Nitrates may be reduced to the more poisonous nitrates and plants containing mustard oil compounds (mustard seed cakes) and Cyanogenetic glucosides (linseed cakes) are liable to liberate, when moistened and left standing, toxic or lethal amounts of mustard oil and prussic acid respectively.

E. Drenching of Animals.

Poultry, Rabbits and Cats.

The plant material is minced when fresh, and ground, when dry, and in order to render it fit for easy passage through thin stomach tubes, it is then forced through a finely meshed sieve by means of a spatula. After this procedure there is very little likelihood of the plant material causing stoppage of the stomach tube. The beaks of birds are easily opened by hand for the introduction of the stomach tube. A safe and easy way of keeping the mouth of a rabbit and cat open is to place a piece of rubber tubing (the same thickness as that used for the stomach tube) behind or over the teeth in the upper and lower jaw, thus pulling the jaws apart. If pieces of string are used instead of rubber tubing, necrosis of the gums will set in when the same animal has to be dosed repeatedly within short periods. Another point of importance is that the material drenched should be continuously shaken during drenching, otherwise the plant material is liable to settle at the bottom of the funnel and block the tube. It is therefore essential that the stomach tube be fixed not to an ordinary glass funnel but to a bulbous container after the fashion of a separating funnel (without the cork used in the separation of the fluids). The stomach tube used in fowls is about 0·5 cm. by 60 cm., whilst that used in rabbits and cats is about 0·75 cm. by 80 cm. The volume of material drenched at a time should not exceed 100 c.c. in poultry and 150 c.c. in cats and rabbits. The volume of material drenched that will be tolerated naturally depends on the size of the animal and on the amount of food present in the stomach (crop).

Dogs and Pigs.

These animals are also drenched when they refuse to take the material fed to them. As dogs and pigs vomit very easily the volume of material drenched, the results of the experiments, especially with irritant substances, should be carefully considered before drawing any conclusions. The plant material is minced or ground and administered through a stomach tube about 1·25 cm. to 1·5 cm. in diameter and about 150 cm. long. An ordinary funnel could be attached to the stomach tube and the material shaken in the flask and poured slowly into the funnel so as to prevent the settling of the plant material at the bottom of the funnel. The pouring of a small quantity of water into the funnel (or bulb) attached to the stomach tube, and allowing this water to run down the tube just as the material to be drenched is poured into the funnel is of great value in preventing blockage of the tube. The volume of material to be drenched again depends on the size of the animal and the amount of ingesta present in the stomach. The mouths of young dogs and pigs can be opened by hand, whilst a piece of rubber covered wood with a central hole (for the passage of the tube) must be introduced cross-wise into the mouths of fullgrown dogs and pigs. Pigs will sometimes drink fluids from horns or old boots from which the points have been removed.

Sheep and Goats.

. The procedure is the same as that described in dogs and pigs. Sheep and goats tolerate large volumes of material and can be drenched more than once on the same day without any apparent ill-effects. The stomach tube should measure 17·5 cm. to 2·0 cm. in diameter and 150 cm. in length. Three litres of material are easily tolerated by unstarved fullgrown sheep and goats. The mouths of these animals are easily opened by hand.

Bovines.

The stomach tube should be 2·25 cm. in diameter and 200 cm. long. Full-grown bovines may receive five litres of fluid without showing any ill-effects. The tongue must be held, and a rubber-covered piece of wood with a central hole must be placed cross-wise in the mouth and the tube passed through the hole.

Equines.

In equines the stomach tube can be passed either through the mouth or through the nose. The latter method is by far the best as the animals often resent the introduction of the gag, which frequently causes damage to the gums. As a rule the introduction of a tube through the nose can be done with very little trouble. Full-grown horses easily tolerate three litres of fluid.

For years the method of drenching animals has been extensively used in all classes of stock, dogs, rabbits and poultry at Onderstepoort. In many cases the same animal has been drenched daily (except Sundays) for months, the stomach tube at times being introduced as often as four times daily and in no case were any deleterious effects noticed both during the period of experimentation and at autopsy.

XVIII. REASONS FOR NEGATIVE RESULTS IN EXPERIMENTS WITH SUSPECTED PLANTS.

From previous discussions some of the reasons for negative results obtained in experiments with suspected poisonous plants are quite obvious, but it seems advisable to mention them again for the sake of completeness. Negative results may have been obtained because A. the plant was non-poisonous; B. non-toxic amounts had been fed or drenched; C. non-toxic parts of the plant had been given; D. the plant had lost its toxicity owing to a change in climatic conditions or after collection; E. the plant was in a non-toxic stage; F. the plant had been fed with too much additional food; G. the experimental animals were not kept under the condition necessary for the production of symptoms (photosensitization, *cynachosis*, *equisetosis*); H. animals which possess a natural or acquired tolerance or immunity to the plant were used in the experiments; I. animals were not kept under observation long enough (plants which cause symptoms of poisoning after a long period of latency); and J. it may have been necessary for the animal to have ingested some other plant along with the poisonous plant before the latter was able to liberate its active principle (for example, a plant may contain a cyanogenetic glucoside, whilst the enzyme necessary for its decomposition may be lacking in the plant concerned but contained in another plant (Finnemore and Cox, 1927).

XIX. REASONS FOR POSITIVE RESULTS IN EXPERIMENTS WITH SUSPECTED PLANTS.

These may be A. the plant is poisonous; B. too large amounts of the plant were introduced into the animal within a certain time; and C. the plant constituents may have changed during the process of preparation for drenching.

A point of importance is that animals fed on one plant only over long periods may develop symptoms of disease which are not due to the presence of poisonous substances in the plant concerned, but which are caused by the absence in the plant of some or other ingredient or ingredients essential for the maintenance of health and life. This is the case with plants deficient in minerals, vitamins, proteins, fats, etc. In addition plants may cause death by virtue of mechanical injury.

XX. LOSSES DUE TO PLANT POISONING.

It is very difficult to obtain reliable figures with regard to losses in stock sustained through plant poisoning as only those cases in which a large number of animals are involved are reported. The state of affairs is not exaggerated when it is stated that in South Africa more stock are lost annually from plant poisoning than from any other disease. The Senior Stock Inspector of Griqualand West reported to the author that *Geigeria passerinoides* Harv. (vermeersiekte) has taken toll of over a million sheep in Griqualand West and the south eastern portions of South-west Africa in the years 1929-1930. In the years 1926-1927 the losses from Tribulosis (geeldikkop) in the north-western Cape Province were calculated to be

between six and seven hundred thousand small stock. In the Uniondale-Willowmore area *Chrysocoma tenuifolia* Berg causes the loss annually of thousands of kids and lambs from *kaalsiekte*. In addition *Dichapetalum cymosum* Hook (gifblaar) and *Urginea burkei* Baker causes heavy losses yearly in stock in the Transvaal, whilst *Senecio spp.* (dunsiekte) have made the breeding of horses impossible in certain parts of the Union of South Africa and Basutoland. In some years wilted grasses (*geilsiekte*, prussic acid poisoning) cause very high mortaily in small stock in the semi-arid regions of South Africa. Tulip (tulp) and *Cotelydon* poisoning is so common that it need hardly be mentioned. Not only in South Africa, but also in America, New Zealand, Australia, Central Africa and India stock poisoning is very common. In Montana and Colorado over two hundred million dollars damage is done to the stock industry by plant poisoning every year.

XXI. ACTION OF POISONS ON FOETUSES.

It is possible for a poison to exert its harmful effects on the wall directly or indirectly through the nervous system of the pregnant uterus or on the foetus or on both. The expulsion of the foetus may be caused by the poison inducing contraction of the uterus or by bringing about the death of the foetus, which is then expelled by the uterus or in both these ways. It is a well-known fact that drastic purgatives very frequently cause abortion.

XXII. INDIRECT POISONING OF HUMAN BEINGS.

Human beings may become poisoned by A. eating the carcases of animals that have died from poisoning, B. drinking, or using in their diet, the milk of poisoned animals; and C. eating honey prepared from the flowers of poisonous plants.

A. Carcases of Poisoned Animals.

The edibility of the carcase, or a part thereof, of an animal which has died from poisoning depends on the channel by which the poison found entrance into the body. If the animals received the poison per os, the flesh can be safely eaten as has been proved repeatedly by a number of investigators. Fröhner (1919, p. 23) states that according to results obtained by his experiments and those of other investigators the flesh (including heart, liver and kidneys) of sheep, rabbits, geese, ducks, fowls, doves and a bovine poisoned with strychnine, eserine, pilocarpine, veratrine, apomorphine, arsenic, oleandrine and lead can be eaten with impunity. Some authors ate the flesh themselves, others fed dogs or examined the flesh for the presence of the poison. Ostertag (1922) also refers at length to the fact that the flesh of poisoned animals could be consumed with safety. It is advisable, as a rule to discard the organs of excretion as here the poison may be found in dangerous amounts. It is for this reason that the liver, kidneys, udder and milk, stomach and intestines should be discarded. If the poison was introduced subcutaneously and intromuscularly as is the case when animals are killed by poisoned arrows, all the flesh, except that part immediately surrounding the point where the arrow struck the animal, is edible.

It would be advisable to discard the organs of excretion mentioned above. Steyn [1931 (*d*)] fed the internal organs and flesh cut from different parts of the body of a sheep killed by the intrajugular injection of strychnine on the left side to dogs. Only those dogs which had received the left front leg, the kidneys and the lungs and heart died, whilst the remaining ones developed no symptoms of poisoning. Henning (1926) was able to produce poisoning in dogs by feeding them on very large quantities of the meat of goats which had died from *Cotyledon wallichii* Harv. poisoning. This is the first record of flesh of animals poisoned per os causing poisoning when eaten. This is possible when the animal, whose flesh is eaten, is much more resistant to the poison concerned than the animal or human being partaking of that flesh. This would mean that the poisoned animal would have such a high concentration of the poison in its flesh, as to cause poisoning in the much more susceptible human being or animal eating its flesh.

B. Milk.

As the lactating mammary gland is an active excretor of many poisons, the milk of poisoned animal or animals treated with very poisonous drugs should be discarded.

Fröhner (1919, p. 24) refers to the young of animals having become poisoned after they have partaken of the milk of animals which had taken arsenic, tartar emetic, meal contaminated with *Agrostemma Githago* Linn, castor bean cakes, and *colchicum*. A native woman who had partaken of coffee poisoned with arsenic, suckled her child, which died from arsenical poisoning, whilst its mother recovered after having exhibited symptoms of poisoning (Juritz, 1910). Muller, Senior Chemical Analyst, fed a suckling cat with arsenically poisoned food with the result that her four kittens died from arsenical poisoning (Juritz, 1910). It stands to reason that the amount of poison secreted in the milk will depend on the amount of poison taken and the time in which it is taken. Many poisons, if not taken in too large quantities at one time, will be changed by the organs, and body tissues and be excreted in harmless forms. Van Itallie (Fröhner, 1919 p. 24) states that morphine, eserine, pilocarpine, iodine, salicylic acid and oil of turpentine are not excreted by the milk.

C. Honey.

Poisoning with honey prepared from the nectar of the flowers of poisonous plants has been known since the earliest ages. According to Leschke (1932a) the first classic description of cases of honey poisoning is to be found in Xenophons Anabasis (Vol. IV, Chapter 8). The symptoms exhibited by the troops who had partaken of poisonous honey were vomiting, diarrhœa, inability to maintain their balance and unconsciousness. Some of the affected cases ended fatally.

Aristoteles, Strabo, Plinius and Dioscorides also refers to cases of poisoning with honey (Leschke, 1932a). Leschke (1932a) furthermore states that honey poisoning has occurred in New Zealand and North America and that the symptoms are those of gastric and nervous disturbances.

It is obvious that no definite symptoms of honey poisoning can be described as these will depend on the effects caused by the plant or plants from which the bees have gathered the nectar.

Pammel (1911) mentions plants from whose flowers it is said poisonous honey is produced. Many cases of honey poisoning are on record. It is said that the bees themselves are poisoned by honey made from the flowers of poisonous plants. In South Africa poisonous honey may be produced from the flowers of *Nerium oleander* Linn, *Datura stramonium* Linn, *Datura tatula* Linn, and other poisonous plants. Pammel says: " A South African species of *Euphorbia* also produces a poisonous honey which was not noted by Greshof ".

SPECIAL TOXICOLOGY.

The following are examples of special toxicology of plants, some of which were investigated by the writer. A comprehensive consideration of the poisonous plants in the Union of South Africa will appear in the contemplated handbook by the writer.

I. GRAMINAE.

THE PROBLEM OF " GEILSIEKTE ".

Many species of the *Gramineae*, some of which constitute our most valuable pasture grasses, develop dangerous amounts of prussic acid (hydrocyanic acid) under certain climatic and soil conditions. This phenomenon has been the cause of severe losses in stock especially in the more arid areas of South Africa. Brown (1864) was the first to refer to " geilsiekte " and subsequently frequent reference to this disease is made in South African Agricultural Journals. The toxicity of wilted pasture grasses has long been known to South African farmers, who wrote to the *Landbou Journal* about " geilsiekte " as early as 1889 (Editorial, 1889), whilst MacOwan (1877) referred to heavy losses caused by this disease in stock. The actual cause of " geilsiekte " due to the ingestion of wilted grasses was investigated by the author in 1929. The term " geilsiekte ", as used by stockwoners, embraces quite a number of diseases in sheep and cattle which are characterised by sudden death. It is, however, a term applied mostly, especially in arid and semi-arid areas, to prussic acid poisoning in sheep caused by the ingestion of certain wilted grasses, and, in other areas by the ingestion of species of *Dimorphotheca* (Steyn, 1929), Henrici (1926) found that certain grasses produced prussic acid very soon after the process of wilting has set in, whilst others do so at later stages of wilting. Those members of the family which have been definitely proved to contain prussic acid under certain conditions will now be discussed.

Anthephora pubescens Nees.

Common names: English—woolgrass; Afrikaans—borseltjiegras, bloubuffelgras.

Distribution: Bechuanaland, Hay, Gordonia, Prieska, Barkly West, Kimberley, Vryburg, Hoopstad, Bloemfontein, Kroonstad, Christiana, Bloemhof, Wolmaransstad, Pretoria, Lydenburg and South West Africa.

Henrici (1926) who used the " vest-pocket test " for prussic acid in her investigations found that when in a wilted state, it contains large amounts of prussic acid. The nature of the cyanogenetic substances present in the wilted grass is unknown. This grass is considered one of the three best pasture grasses in those areas where it occurs (Burtt-Davy, 1912).

Aristida congesta R. & S.

Common name: Afrikaans—steekgras; Suto—*phutha-dikxoba, mahlaswa.*

Distribution: Common all over Union of South Africa.

This grass when wilted contains large amounts of prussic acid, (Henrici, 1926).

Aristida uniplumis Licht.

Common names: English—large Bushman grass, shiny grass; Afrikaans—langbeen t'waa (Toa) gras (southern Bechuanaland).

Distribution: Very common in southern Bechuanaland, extending westward to Namaqualand (Burtt-Davy, 1912), also in Griqualand West, Transvaal, southern Orange Free State, and certain parts of the Karroo (Colesberg).

It yields prussic acid when wilted (Henrici, 1926).

Eustachys paspaloides (Vahl) Lanza & Matti

[=*Chloris petraea Thunb.* (*non Swartz*).]

Common names: Suto—sebokyuyana.

Distribution: Common all over Union of South Africa.

Rosenthaler (Wehmer, 1929) records 0·002 mg. per cent. prussic acid in the leaves while Henrici (1926) found prussic acid in both fresh and wilted specimens.

Raphis montana var. tremula (Stapf.) Phill.

(=*Chrysopogon serrulatus* Trin. =*Andropogon monticola var. Trinii Hook. f.*).

Common names:

Distribution: Pretoria, Waterberg, Bloemhof, Bechuanaland, Hopetown.

When wilted it yields prussic acid (Henrici, 1926).

Cynodon Bradleyi Stent.

Common name: English—Bradley grass.

Distribution: Cultivated as a lawn grass.

Specimens of this grass collected from a lawn at Onderstepoort yielded large amounts of prussic acid when wilted (Steyn, 1929). On one occasion even fresh (unwilted) specimens, collected in the early morning (about 8 a.m.) within two days after a heavy rain, showed

the presence of prussic acid. Subsequent tests performed with fresh specimens failed to reveal the presence of prussic acid. Sundried specimens collected when wilted and tested two days after picking, still contained large amounts of prussic acid.

Cynodon dactylon Pers.

Common names: English—Florida grass, Germiston grass, Bahama grass, Scotch grass, Devil's grass, Bermuda quick grass, Bermuda grass, Dub grass, Doab grass, fine couch grass; Afrikaans—Batawiese kweek, fynkweek, kwaggakweek, Oost-indiesekweek, kruisgras, kweek; Suto—*mohlwa, morara;* Xosa—*uQaqaqa.*

Distribution: Occurs practically throughout the Union of South Africa. Common on old lands near homesteads and "kraals".

Wehmer (1929) refers to this species, but does not mention anything about the presence of prussic acid. Specimens of this plant growing at Onderstepoort were tested by the author and were sometimes found to contain small amounts of prussic acid when in a perfectly fresh (unwilted) state and invariably very large quantities when wilted during periods of hot dry weather.

Cynodon incompletus Nees.

Common names: English—quick grass; Afrikaans—regte kweek, Transvaal kweekgras.

Distribution: Albany, Uitenhage, Queenstown, Humansdorp, Hanover, Port Elizabeth, Griqualand West.

Wehmer (1929) states it contains prussic acid, but not constantly.

Cynodon transvaalensis Burtt-Davy.

Common names: English—quick grass; Afrikaans—kweekgras, kweek.

Distribution: Ermelo, Vereeniging, Witwatersrand, Kroonstad and Bloemfontein.

At Onderstepoort the author found that wilted specimens contain large amounts of prussic acid.

Digitaria eriantha Steud.

Common names: Suto—*mmoyane.*

Distribution: Bechuanaland, Eastern Cape Province, Griqualand West, Prieska, Tembuland, Natal.

Henrici (1926) found prussic acid in wilted specimens.

Pogonarthria squarrosa (Licht) Pilger

(= *Pogonarthria falcata* Rendle).

Common names: Suto—*mongoyane.*

Distribution: Bechuanaland, Natal, Basutoland, Orange Free State, Griqualand West, Transvaal.

Wilted specimens contain prussic acid (Henrici, 1926).

Sorghum verticilliflorum Stapf.

[=*Andropogon halepensis* Brot. var. *effusus* Stapf].

[=*Sorghum halepense* Nees (non Pers)].

Common name: English—Johnson grass.

Distribution: Cultivated as a fodder crop.

According to Wehmer (1929) and Cough (1932) *Sorghum halepense* (Johnson grass) may develop dangerous amounts of prussic acid. Mathews (1932) reports seven cases of poisoning with "*Sorghum halepense*" in cattle. Periods of drought varying from days to weeks preceded every outbreak. At the time of the outbreaks the grass was wilted and showed sunburnt tips. The symptoms described markedly resemble those in prussic acid poisoning. Burtt-Davy (1903-1904) states that "Johnson grass or Evergreen Millet (*Andropogon halepensis* var. *effusus* Stapf) is one of the most pernicious weeds ever introduced by human agency". According to him Johnson grass has found its way into the Transvaal, and is now a weed at the Potchefstroom Agricultural College.

Sorghum saccharatum Pers.

Common names: English—Sorghum; . Afrikaans—Soetriet; "Imphee".

Distribution: *Cultivated.*

When wilted it contains dangerous amounts of prussic acid, which is liberated from a cyanogenetic glucoside (Wehmer, 1929).

Sorghum Sudanense Stapf.

Common names: English—Sudan Grass; Afrikaans—Sudangras.

Distribution: Cultivated as a fodder crop.

It is well known that Sudan grass may under certain climatic and soil conditions develop fatal amounts of prussic acid. The following is the summary of a publication by Swanson (1921), who investigated the toxicity of Sudan grass:

"(1) Hydrocyanic acid was found in large amounts in Sudan grass used for pasture and no harm resulted to cattle.

(2) Liberation of hydrocyanic acid from Sudan grass is apparently associated with enzyme action. Digesting in water at room temperature for several hours and then distilling gave larger amounts of hydrocyanic acid than if sulfuric acid was added at once. Hot water and dry heat diminished the amount of hydrocyanic acid obtained. Slow drying caused the hydrocyanic acid to disappear. Tests made on wilted samples or those several days old may be worthless.

(3) Making Sudan grass into silage did not diminish the amount of hydrocyanic acid.

(4) Tests made immediately on frosted Sudan grass gave very large amounts of hydrocyanic acid, but it disappeared rapidly as soon as the plant began to wilt; when dry the hydrocyanic acid had disappeared.

(5) While Sudan grass giving a strong test for hydrocyanic acid was not harmful to cattle under other conditions it was harmful. Immunity was not due to habituation ".

According to Horwath (1931) sheep grazing on Sudan grass developed symptoms of photosensitization. Photosensitization due to the ingestion of this grass has not as yet been noticed in South Africa, where conditions appear to be most favourable for the development of this malady.

Sorghum vulgare Pers.

Common names: English—kaffir-corn; " Dhurra "; guinea-corn; Indian millet; great millet; Juár of India, Durrha Shirshabi of Egypt. Afrikaans—kafferkoring. Zulu—*ama Bele.*

Distribution: Widely cultivated in South Africa, especially by the natives, as a food grain. Its toxic properties have been known for a long time.

Dunston and Henry (1902) isolated a cyanogenetic glucoside (dhurrin) from kaffir-corn. The interaction of dhurrin and an enzyme, which is present in the plant and apparently is identical with the emulsin of bitter almonds, liberates prussic acid, dextrose and para-hydroxybenzaldehyde. Dilute acids have the same action as this enzyme on dhurrin. Dhurrin is resolved into dhurrinic acid and ammonia when heated with alkalis. Dunston and Henry showed that only the young plant, and not the mature plant and " seeds ", contain dhurrin or prussic acid. Robinson (1930) also refers to the toxicity of immature kaffircorn plants. •The disappearance of dhurrin and prussic acid from the cut plant apparently occurs very slowly as cattle died from eating the plant three days after it had been cut.

Sporobolus fimbriatus Nees.

Common names: Suto—*matulo-a-maholo.*

Distribution: Southern Bechuanaland, South Eastern Cape Province, Basutoland, Orange Free State, Transvaal.

Henrici (1926) demonstrated the presence of prussic acid in the wilted plant.

Themeda triandra Forsk.

Common names:

Distribution: Throughout Union of South Africa.

According to Henrici (1929) it contains prussic acid when wilted.

Henrici (1926) failed to demonstrate under any circumstances the presence of prussic acid in the following grasses: *Eragrostis superba* Peyr., *Eragrostis lehmaniana* Nees, " *Cymbopogo plurinodis* ", and

"*Fingerhuthia africana*". Fresh specimens of *Pennisetum claudestinum* Chiov (kikuyu grass) and specimens in all stages of wilting up to complete withering were examined at Onderstepoort by the author for the presence of prussic acid with negative results.

Zea mays L.

Common names: English—maize, mealie, Indian corn ; Afrikaans—mielie; Sesuto—*poone.*

Distribution: Extensively cultivated as a foodstuff for man and animal.

Walsh (1909) states that the male inflorescence contain a variable amount of prussic acid. Burtt-Davy (1912) (pp. 189-190) writing about the American cornstalk disease refers to a letter received from a correspondent. The latter states that during an exceptionally dry year thousands of head of cattle were lost in the cornfield of Western Nebraska. Upon investigation it was found that the mealie stalks contained a large percentage of prussic acid. The correspondent however adds: I am not positive if I am correct in the poison ". Quite a number of publications on cornstalk disease are mentioned by Burtt-Davy. Price and Craig (Burtt-Davy, 1912) consider it possible that cornstalks may at times contain prussic acid. According to a verbal report made to the author a number of donkeys, which had been driven on to a land of wilted mealies in Natal, died within a few hours.

Experiments conducted by the author at Onderstepoort have shown that under certain soil and climatic conditions green mealies may develop prussic acid. The results were however by no means constant. A large number of tests were conducted on wilted mealies growing on black clay soil and sandy soil and only in a few instances was prussic acid detectable and then only in the specimens growing on red sandy soil.

Toxicity of the Cyanogenetic Gramineae.

The toxicity of the above-mentioned cyanogenetic Gramineae obviously depends on their cyanogenetic glucoside or prussic acid content, which varies considerably according to climatic and soil conditions, and upon the rate at which such plants are ingested. Furthermore, the toxicity of plants containing cyanogenetic glucosides depends to a considerable extent on the amount of enzyme, which is capable of splitting up these glucosides, present in the plant. The fact that such enzymes may be contained in other plants eaten by stock should not be lost sight of. Seddon and King (1930) state that fresh plants containing 0·02 per cent. of prussic acid and dry plants containing 0·05 per cent. of prussic acid must be considered dangerous, assuming that a sheep eats an average of 500 grams of the fresh plant.

The following conditions may give rise to the formation of fatal amounts of cyanogenetic glucosides (or prussic acid) in the above-mentioned plants: wilting and withering, especially during spells of hot dry weather; disease (Willaman and West, 1916); frost; bruising; trampling; soils of different composition (plants grown on good

soil contain more prussic acid than those grown on poor soil and fertilizing with nitrates stimulates the production of prussic acid (Burtt-Davy, 1912; Cough, 1932). The younger and the more succulent the plants are the more likely they are to produce lethal amounts of prussic acid. During the process of desiccation most, if not all, of the prussic acid disappears from the plants. Well cured plants, which contained dangerous amounts of prussic acid at the time of cutting, can as a rule be fed to stock without any danger of poisoning. The rate of drying of plants, which contain prussic acid or cyanogenetic glucosides, is of great importance. Swanson (1921), who investigated the amount of prussic acid present in Sudan grass from the time of cutting up to complete dryness states: "The portion tested at once gave large amounts, that dried in the oven somewhat less, that dried in the sun still less, and that dried slowly in the shade none or only a trace". Rosenthaler (1929) found that a decrease in the intensity of light is accompanied by a decrease in the prussic acid content of leaves of "*Prunus laurocerasus*". Before feeding hay prepared from mealie stalks and sorghums it would be advisable to collect specimens from various parts, and especially from the centre, of the stacks (or silos) and submit these to prussic acid tests.

The possibility of a plant containing a cyanogenetic glucoside but not the necessary enzyme to split it should be mentioned here. This enzyme might, however, be present in some other plant. This was found to be the case in Australia with *Eremophila maculata* Fr. and *Acacia Georgina* Bailey (Finnemore, 1931).

The Physiological Significance of Cyanogenesis.

Prussic acid was discovered by a Swedish chemist in 1782. The production of this acid by a plant was first recorded by Böhm in bitter almons in 1802 (Heffter, 1923).

In considering cyanogenesis in plants we have to distinguish between those plants (*Dimorphotheca spp.*) which normally contain prussic acid (or cyanogenetic glucosides) and those (wilted grasses) which develop prussic acid under certain conditions only.

There is no consensus of opinion with regard to the physiological significance of cyanogenesis in plants and what has been said under "VII. The Toxic Principles of Plants and their Physiological Significance" also applies to cyanogenesis in plants.

Dunston and Henry (1906), Gresshof (1906), Willaman and West (1916), Czapek (1921), Henrici (1926) and Robinson (1930) refer to the physiological significance of cyanogenesis in plants.

Bach (Dunston and Henry, 1906) "supposed that from the small amount of nitrate present in cell-sap, nitric acid was liberated in minute amount by the considerable quantities of oxalic and carbonic acids usually present, and that this free nitric acid was continuously reduced by formaldehyde, producing hydroxylamine, which immediately combined with formaldehyde, forming formaldoxime. The latter might undergo transformation in two ways. It might be converted into the isomenic formamide, which by simple

dehydration would give prussic acid and water, and in this way account for the frequent occurrence of this acid in plants, or the formamide might be hydrolysed, yielding ammonium formate, so supplying ammonia and formic acid ". This view of Bach seems to offer quite a feasible theory of cyanogenesis in wilted grasses, as in this condition natural dehydration occurs, thus causing the formation of prussic acid from a large proportion of the formamide present in the wilted plant. Gautier (Dunston and Henry, 1906) holds: " that the free nitric acid of cell-sap reacts with formaldehyde, forming free prussic acid, carbon dioxide, and water ".

Willaman and West (1916) consider the large amount of prussic acid in " *Sorghum* ", when the water supply is inadequate, a result of the lack of glucoside stimulation. The increased amount of prussic acid in " unhealthy Sorghum plants " is thought by Willaman and West (1916) to be produced for the purpose of stimulating hormones.

The view most widely held at present is that prussic acid is an intermediate product of proteid synthesis, hence plants will contain most of this acid when protein metabolism is most active. Treub (Robinson, 1930) regards the following facts as a support of this view:—

(*a*) The occurrence of cyanide in phloem and pavicycle (Pangium edule); (*b*) the absence of protein from your " special cells ", the basilar hair cells and the oxalate-containing cells (" Pangium edule "); and (*c*) the disapperance of cyanide from leaves during senescence, or when the plant is kept in the dark. The accumulation of cyanide in the basilar hair cells and the oxalate containing cells was an indication to Treub that its formation was normally related to photosynthetic processes.

Fearon (1926) discusses the possibility of cyanic acid being an intermediate in the urea-urease system.

Absorption, Mode of Action, and Elimination of Prussic Acid.

Prussic acid is absorbed by the blood vessels and not by lymph vessels; tendons aponeurosis and nerves absorb prussic acid very slowly (Heffter, 1923). The action of prussic acid is most pronounced when inhaled or when injected intracheally, death occurring within a few seconds. Blake (Heffter, 1923) found that prussic acid is not absorbed from the stomach when the vena portal is ligatured. Symptoms of poisoning, however, set in within a minute after removal of the ligature. Bonanni and Marino (Heffter, 1923) maintain that prussic acid is absorbed by the oesophagus. It is quite conceivable that prussic acid being a gas will diffuse not only through all mucous membranes but also through the skin. Theben and Coullon (Heffter, 1923) found that prussic acid is less poisonous when given per os than when administered rectally. The conjunctiva is a very active absorber of prussic acid.

When administered per os the largest percentage of prussic acid will be present in the blood and intestines, whilst when injected intraveneously the largest proportions are found in the heart and brain (Heffter, 1923).

A certain percentage of the prussic acid taken is converted into the relatively non-toxic sulphocyanate and excreted as such in the urine, whilst some is eliminated as such by the lungs. It is held that the sulphur necessary for the formation of sulphocyanate is derived from protein. The bright red colour of the venous blood is due to the fact that prussic acid paralysis the oxidative enzymes, which are responsible for the transference of oxygen from the red blood corpuscles to the tissue constituents (internal asphyxia). If the affected subject survives the effects of prussic acid poisoning for a time the blood turns cyanotic owing to inhibition of respiration and the consequent small intake of oxygen. In cold blooded animals the reddish colour of the blood persists from half-an-hour to twenty-four hours after death. Prussic acid is an active poison of the nervous system causing first stimulation and later on paralysis of the centres in the medulla oblongata, namely, the centre of respiration and the vasomotor centre, and also the motor centres in the brain. According to Voigt (1932) the origin of the spasms caused by prussic acid in the ventral horns of the spinal cord. The disturbances in the blood circulation are due to actions on the sinus nodes.

Toxicity of Prussic Acid.

When inhaled the toxicity of any gas depends on the time of inhalation and on the concentration of the gas in the inhaled air. The toxicity of gases may be expressed by concentration-time curves as was the general practice in the Great War. When taken per os the concentration (amount of prussic acid or cyanogenetic glucosides) in the materials ingested and the time taken to ingest such materials are as is usually the case with toxic substances, the factors which determine the toxic and lethal doses of cyanogenetic substances. The following table is taken from Barcroft's publication (1931) with regard to the toxicity of prussic acid when inhaled:—

Animals in Order of Sensitiveness to Prussic Acid.

Animal.	Lethal time of exposure to a concentration of 1·0 mg/litre (minutes).	Animal.	Highest approximate concentration which can be breathed indefinitely mg/litre.
Dog.......	0·8	Dog.......	0·10
Mouse.....	1·0	Rat.......	0·10
Cat.......	1·0	Mouse.....	1·14
Rabbit....	1·0	Rabbit....	0·18
Rat.......	2·0	Monkey....	0·18
Guinea-pig.	2·0	Cat.......	0·18
Goat......	3·0	Goat......	0·24
Monkey...	3·5	Guinea-pig	0·40

With regard to the susceptibility of man to prussic acid Barcroft (1931) states that man is not very susceptible in comparison with the dog. He also states that canaries and pigeons are extremely susceptible to prussic acid poisoning, a concentration of 1:10,000 killing the former within two minutes, whilst pigeons vomit at 1:10,000 and die in 1:5,000. Schütze (Petri, 1930), in the course of experiments upon animals and human beings, found that highly concentrated prussic acid is absorbed by the intact skin.

The following are the lethal doses of prussic acid and its salts administered in one dose to animals, as computed from the results of experiments conducted by the author at Onderstepoort and from the publications of Fröhner (1919), Lander (1926), Hindmarsh (1930), Leschke (1932), and Voigt (1932): Horses 0·5-1·0 gm. HCN per os; 4·0-8·0 gm. KCN per os (approximately 0·009 gm. per Kg. body-weight); cattle: 2·2 mg. HCN per Kg. body-weight intraperitoneally; sheep: 2·2 mg. HCN per Kg. body-weight per os; 6·0 mg. KCN per Kg. body-weight per os; dogs: approximately 2·0 mg. KCN per Kg. body-weight; small animals (birds, guinea-pigs): approximately 0·1 mg. HCN per animal; rabbits: 12·0-15·0 mg. KCN per Kg. body-weight; frogs: 0·5 mg. NaCN per gm. body-weight (injected into lymph sack); white mice: 0·25 mg. NaCN per mg. body-weight injected subcutaneously).

According to Kobert (1902) 0·15 gm. chemically pure KCN and seventeen drops of oil of bitter almons taken per os are sufficient to cause death in adult human beings, whilst Leschke (1932) states that 0·1-0·2 gm. KCN causes death in human beings within twenty to forty minutes. The amount of acid in the stomach naturally plays an important rôle in the determination of the toxicity of potassium cyanide.

Prussic acid being a diffusible gas is very rapidly absorbed from the gastro-intestinal tract and is also eliminated at a very rapid rate by the lungs and skin. Animals poisoned with just sublethal amounts of prussic acid will be found to show symptoms of dyspnoea only within a very short time after having been at the point of death.

Blake (Glaister, 1931) " has demonstrated that if prussic acid be introduced into the stomach of an animal by a fistulous opening, after ligature of the portal vessels, no poisonous results will ensure; but that immediately after removal of the ligatures, the poison begins to act ".

Symptoms of Prussic Acid Poisoning.

(*a*) *Acute poisoning.*—The symptoms vary according to the size of the dose of prussic acid and mode of application. It is a poison which acts extremely rapidly, large amounts causing death almost instantaneously with spasms and respiratory paralysis. Smaller doses of prussic acid cause accelerated and deepened respiration, accelerated, irregular and weak pulse, bright-red mucous membranes, which later turn purplish in colour on increased salivation and frothing at the lips, muscular twitchings, shivering, staggering, as if intoxicated and dropping down, staring and anxious look in the eyes, dilatation of the pupils, clonic spasms of the neck and legs, especially front legs, pronounced bloating, distress, epileptiform convulsions at varying intervals, opisthotonus, orthotorus, trismus, coma and death due to respiratory paralysis. The heart usually continues to beat for some time after respiration has stopped.

(*b*) *Chronic poisoning.*—According to Kobert (1902) the following symptoms and lesions may be encountered in chronic prussic acid poisoning in human beings: Dyspnoea, lassitude, degeneration of the muscles of the extremities, headaches, gastro-intestinal

disturbances, uncertain gait and difficulty in moving the jaws; when the gas is inhaled hyperaemia of the pharynx mucosa, severe irritation in the throat, pronounced salivation, retching, vomiting, headache, bradycardia, lassitude and albuminuria may be experienced.

There is no concensus of opinion with regard to chronic prussic acid poisoning. The author was unable to produce chronic poisoning in sheep and rabbits by drenching these animals at twenty-four hourly intervals with toxic but not lethal amounts of potassium cyanide for periods up to one month. Heffter (1923) however states that repeated administration of prussic acid causes chronic poisoning but not always with characteristic symptoms. He states that frequently the symptoms resemble those of a cumulative action. According to Koelsch and Seligmann (Petri 1930) continuous action of prussic acid on human beings causes oedematous swellings of external genital organs, urticaria-like eruptions on the skin, eczemata, and itching nodules and vesicles. Haemorrhages are rare. According to Koelsch chronic prussic acid poisoning causes the development of "Acna rosacea", an angioneurotic inflammation. The amount of prussic acid given and the interval at which it is administered will naturally determine whether repeated administration will have any effect on the system.

Post-mortem Appearances.

In peracute cases of prussic acid poisoning the blood is bright-red owing to the formation of cyan-haemoglobin. The bright-red colour of the venous blood is due partly to the ineffective deoxidisation of the arterial blood in the tissues. On opening up the body cavities and stomach (rumen) a smell of bitter almonds is perceived provided the post-mortem is performed soon after death.

The venous system is distended and if animals are skinned soon after death cyanotic blood, the coagulation of which is retarded, escapes freely from the subcutaneous tissues. As a rule the right ventricle of the heart is markedly distended. The stomach (rumen) shows pronounced distension with gas and there is marked hyperaemia and sometimes oedema of the lungs and liver. There may be haemorrhages in the serous membranes.

Staemmler (1932) conducted an autopsy on a human being four days after death had occurred from poisoning with potassium cyanide. In spite of very hot weather there were hardly any signs of decomposition. A distinct smell of oil of bitter almond was emitted from the mouth, and the stomach contents smelled of ammonia and of oil of bitter almond. The mucosa of the stomach and of the anterior portion of the small intestine was swollen, reddish in colour and covered with a mucous substance. The consistence of the affected mucosa was peculiarly firm. Histologically no changes, apart from slight dilation of the bloodvessels, could be detected. A remarkable finding was that practically no post-mortem changes were noticeable in the affected mucosa. This was borne out by the fact that the cell nuclei stained perfectly well. Staemmler ascribes the preservative effect of prussic acid to the fact that it is a specific poison for oxidising ferments.

It is interesting to note that at the time the autopsy was conducted prussic acid was still detectable in the gastric contents.

Histology.

Petri (1930) discussed the histology of prussic acid poisoning. If the gas is inhaled the erythrocytes assume the form of the fruit of *Datura stramonium* Linn. (*Stechapfelformen*) and are ultimately completely destroyed. In chronic poisoning there is high haemoglobin content owing to an increase in the number of erythrocytes, lymphocytosis, an increase in the immature and mature basophiles.

There is oedema and pronounced venous congestion of the central nervous system with haemorrhages in the subdural tissues and in the pia mater. Incipient "inflammation" in the pallidum, spinal cord and medulla oblongata, acute affection of the ganglian cells and small vessels occluded by hyalin thrombi are also mentioned.

In chronic poisoning, animals which die with symptoms of atrophic paralysis show chromatolysis, vacuole formation, shrinking, and dissolution of the protoplasm of the cells of the ventral horns of the spinal cord. In rabbits there is "degeneration" of the peripheral nerves. The small brain vessels show fatty changes and calcification, and the lungs may show haemorrhages in the parenchym. Extensive haemorrhage into the fatty tissues of the pancreas is also described.

Diagnosis of Prussic Acid Poisoning.

The symptoms and post-mortem appearances must be considered in the diagnosis of prussic acid poisoning, as the presence of small amounts of prussic acid in the gastro-intestinal contents does not justify a definite diagnosis of poisoning by this acid. The amount of prussic acid present in the gastro-intestinal contents, blood and organs depends on (*a*) whether free prussic acid or its combinations are concerned, (*b*) the method of administration, (*c*) the course the poisoning has taken, and (*d*) the time that has elapsed since death has occurred.

Prussic acid disappears from the carcase more rapidly than its salts and cyanogenetic glucosides. Seni and Revello (1929) who conducted experiments upon dogs with gaseous prussic acid and aequeous solutions of prussic acid and potassium cyanide, found that if the minimum lethal dose of prussic acid is given this poison cannot be recognised either by chemical or spectroscopic means. When three times the minimum lethal dose is given the prussian blue reaction is positive, whilst the spectroscopic examination is still uncertain. When large doses of prussic acid are given, this poison disappears from the organism within twenty days. In the case of poisoning with gaseous prussic acid the tests for this acid are invariably negative. With regard to the detection of prussic acid in carcases of rabbits poisoned with *Dimorphotheca spectabilis* Schltr, which contains a cyanogenetic glucoside, Steyn (1931A) found that when the minimum lethal dose of this plant was given no prussic acid was detectable in the gastro-intestinal contents forty-eight hours after death, whilst the test for prussic acid was positive when large

amounts of the plant were given. A rabbit drenched with a sublethal quantity of *Dimorphotheca spectabilis* Schltr., which had developed symptoms of poisoning, showed no prussic acid in the stomach and intestinal contents within five hours after dosage. The stomach contents of a rabbit, that had died from poisoning by this plant, was kept in a well stoppered bottle and up to four months after collection prussic acid was still detectable. No more material was left for further tests. In the above experiments the " vest-pocket test ", described below, was used for the detection of prussic acid.

From the above experiment it appears that gastro-intestinal contents containing hydrocyanic acid can be kept for long periods without any risk of the hydrocyanic acid escaping, provided the vessel in which such contents are placed is air-tight. Furthermore in the light of the foregoing experiments, it is proposed to adopt the following procedure in attempting to diagnose cases of prussic acid poisoning: About 4 ounces of the stomach contents of the animals suspected to have died from " geilsiekte " should be collected as soon as possible after death. These must be placed immediately in a container (preferably a fruit jar fitted with an unperished rubber ring) which should be firmly closed so as to prevent the escape of gases. The specimen should then be packed carefully so as to avoid breakage, and be sent to a laboratory for a chemical test. In each case the time which has elapsed between the death of the animal and the taking of the specimen should be stated. In this way it will be possible to ascertain whether a disease thought by the farmer to be " geilsiekte " is hydrocyanic acid poisoning or some other disease of unknown aetiology.

Vest-pocket Test for Cyanogenesis.

This test is eminently suitable for work conducted under field conditions. The following description of the test is taken from a publication of Henrici (1926, pp. 495-496):—

" Picrate solution and papers, 5 gm. sodium carbonate and 0·5 gm. picric acid in 100 c.c. water. Wet ordinary filter paper with this, hang up to dry until only just ' perceptibly moist ', and cut into convenient strips, about 1 cm. by 4 cm. Papers should be made up fresh every week as sensitiveness decreases with time. The solution keeps well for months in a stoppered bottle.

" Test—into a stout glass tube, about 1½ cm. of 7 cm. or other convenient vest-pocket size, push a few grammes of the moist shredded plant (or moist pulverised seed). Add two or three drops of chloroform to hasten autolysis, insert a slip of ' perceptibly moist ' picrate paper at the top, and cork tightly. Incubate in a vest-pocket, examining at intervals. Liberation of HCN is indicated by reddening of the yellow picrate paper—within a few minutes if the amount is large, after twenty-four hours if only traces are present. If the paper remains lemon-yellow it either means that a cyanogenetic glucoside is absent or that a hydrolytic enzyme is not intimately associated with it. In the latter case chemical analysis may still show hydrocyanic acid, but with the majority of plants analysis will not show much if the simple test fails. It may be added that the test

is so delicate that cyanogenesis is revealed in a large number of common non-toxic edible plants, an easily comprehensible fact in view of the significance of cyanogen in normal plant anabolism."

Further tests for the presence of prussic acid in the gastro-intestinal contents, blood and organs, and in plants are described by Koert (1902), Furlong (1914), Fröhner (1919), Swanson (1921), Lander (1926), Glaister (1931) and Leschke (1932).

Differential Diagnosis.

The presence of prussic acid in the carcase will assist to a considerable extent in the diagnosis of prussic acid poisoning. In cases which simulate prussic acid poisoning but which yield negative results for the specific reaction, the circumstantial evidence, symptoms, post-mortem appearances, and chemical tests may be of value in diagnosing the cause of death.

Treatment.

Prussic acid is a rapid acting and deadly poison and unless treatment applied before serious symptoms of poisoning have developed, it will be of very little or no avail. Treatment may be administered on the following lines. In human beings and those animals that are capable of vomiting, emetics will be of value when unabsorbed prussic acid or its combinations are still present in the stomach. In such cases stomach lavage with the chemical antidote mentioned below will materially retard or prevent further absorption of the poison. Bleeding and blood transfusions, undoubtedly will be of value in the treatment of prussic acid poisoning.

As pharmacological antidotes (symptomatic treatment), the following drugs may be given: Ether subcutaneously, camphor, caffeine, veratrin, strychnine, alcohol (brandy) per rectum, lobeline subcutaneously and sodium nitrite intravenously. Lobeline is a valuable stimulant of the respiratory centre, and is preferable to atropine. It is held that nitrates react with urea in the body producing carbon dioxide, which stimulates respiration. The intravenous dose of sodium nitrite for a human being is 10-20 c.c. of a 1 per cent. solution (Barcroft, 1931). Brooks (1932) suggests that methylene blue injections could be used advantageously in prussic acid and carbon monoxide poisoning, as it activates the oxygen supply of the body. Geiger (1932) reports favourably on the use of methylene blue (methylthionine chloride, U.S.P.) in prussic acid poisoning in a human being. The patient had received 50 c.c. of a 1 per cent. sterile aequeous solution of this preparation intravenously with the result that complete recovery occurred within fifteen minutes. Artificial respiration, oxygen inhalations, cold affusions and electrical stimulation of the phrenic nerves and chest are of great value. Adrenalin will retard absorption from the gastro-intestinal tract. The following may be administered as chemical antidotes: Sodium thiosulphate, colloidal sulphur, ferrous sulphate followed by a solution of potassium carbonate, glucose, dioxyacetone, glycerinaldehyde, potassium permanganate, hydrogen peroxide and ammonia. Prussic acid and its salts form sulphocyanic (thiocyanic) and sulphocyanides (thiocyanides) with sodium thiosulphate ($NaCN + Na_2S_2O_3 + O - NaCNS + Na_2SO_4$). In the blood, which is alkaline, liberation of

sulphur from sodium thiosulphate is slower than in acid medium, on the other hand alkalinity favours the formation of sulphocyanic acid and sulphocyanides. Sodium thiosulphate is therefore of greater value as a preventive than as a curative of prussic acid poisoning. It can be administered in the following doses: Human beings—0·1-0·2 gm. intravenously; 0·65-2·0 gm. per os; animals—0·003 gm. per Kg. body-weight intravenously and about five times this dose per os (Kobert, 1902; Lander, 1926; Forst, 1928; Milanesis, 1929; and Leschke, 1932). Chistoni and Foresti (1932) found that tetrathionate of sodium ($Na_2S_4O_6$) has a marked antidotal action on prussic acid, causing formation of the harmless alkaline sulphocyanate. This action of tetrathionate of sodium is also exerted on prussic acid present in the tissues, hence this sodium compound is of great value in the treatment of cases of prussic acid poisoning already showing symptoms of poisoning. Sulphides will have effects similar to those described above on prussic acid poisoning.

The sulphur (sulphide) of protein also combines with prussic acid, the combination is however very slow. With regard to sulphur metabolism in prussic acid poisoning Kahn and Goodridge (1926, p. 373) write: "Loewy, in 1907, demonstrated that hydrocyanic acid not only increased the protein catabolism, but also influenced the metabolism qualitatively. Wallace and Richards studied the effect of potassium cyanide upon metabolism, and they observed that the total S-output was increased on the day of poisoning, but, unlike the total nitrogen, it fell on the following day. The neutral sulphur fraction was increased, whereas the sulphate sulphur was diminished, showing that the oxidative processes in the body were lessened.

Loewy, Wolf and Osterberg concluded from their experiments on dogs that even in slight cases of poisoning with hydrocyanic acid there was an appreciable increase in the neutral sulphur in the urine and with marked poisoning the neutral sulphur fraction was greater than the sulphate sulphur fraction. It is therefore clear that hydrocyanic acid lessens the exidative processes in the body ".

According to Kahn and Postmonteir (Denis and Reed, 1926-7) the non-protein sulphur in the blood exists in three forms, namely, inorganic, ethereal and neutral. There are also sulphur-containing lipoids in animal tissues (Denis and Reed, 1926-7).

When a certain percentage of cyanide is administered to dogs the neutral sulphur of the urine is increased absolutely and relatively (Smith and Malcolm, 1930).

Prussic acid forms innocuous ferrocyanides with ferrous salts in concentrated alkaline solutions. It is therefore advisable to administer freshly-prepared ferrous hydrate *ad lib.* This antidote is only of value as far as the prevention of absorption of prussic acid or cyanides from the gastro-intestinal tract is concerned (Kobert, 1902; Fröhner, 1919; Lander, 1926; Couch, 1932).

Glucose (dextrose) and its decomposition product dioxyacetone proved to be of great value as antidotes in the treatment of prussic acid or cyanide poisoning (Forst, 1928; Casser, 1930; Barcroft, 1931; Wiegand, 1931; Couch, 1932; Forst, 1932; Leschke, 1932). These

and other carbohydrates form innocuous cyanhydrin with prussic acid. According to Forst (1932) cyanhydrin is then slowly decomposed in the system again liberating prussic acid. The liberation of prussic acid from the carbohydrate cyanhydrin depends to a certain extent on the rate of oxidation of the carbohydrate. The most effective treatment of cases of prussic acid or cyanide poisoning apears to be combined intravenous injections of dioxyacetone and colloidal sulphur (Forst, 1928). Glucose may be administered orally, tntramusculary intravenously, intraperitoneally, subcutaneously or rectally. For intravenous administration glucose is dissolved in distilled or physiological salt solution and sterilised before injection. The intravenous dose of a 50 per cent. solution of glucose for man and animal is about 1·0 c.c. per Kg. body-weight. Rabbits may receive about 2-4 c.c. per Kg. body-weight. For subcutaneous injection a 10 per cent. and for intraperitoneal injection a 5 per cent. solution is recommended (Milks, 1930). Dioxyacetone in a 20 per cent. solution in physiological salt solution [Oxantin (Hoechst)] could be administered intravenously in half the doses prescribed for glucose. Glycerinaldehyde also converts prussic acid into the comparatively non-toxic glycerinaldehyde cyanhydrin.

Potassium permanganate administered in solutions up to 0·5 per cent. in strength destroys by oxidation the prussic acid still present in the gastro-intestinal tract (Kobert, 1902; Fröhner, 1919; Couch, 1932; Leschke, 1932). Hydrogen peroxide causes the formation of comparatively harmless oxamide $\frac{(H_2O_2 + 2\ HCN - CONH_2).}{CONH_2}$ The subcutaneous injection of hydrogen peroxide in a 3 per cent. solution is recommended and also a stomach lavage (Kobert, 1902; Fröhner, 1919; Couch, 1932; Leschke, 1932).

Ammonia inhalations are recommended as ammonia has a strong stimulating action and has a tendency to convert prussic acid into less dangerous substances (Couch, 1932).

Prevention of Prussic Acid Poisoning.

Keeser (1930) found that rabbits fed with green feed and ferrous chloride showed a greater resistance to cyanide poisoning than those rabbits fed on milk rice or green feed only. Keeser concludes that this increased resistance in the animals which received ferrous chloride, is due to the increased iron content of the tissues.

Feeds rich in carbohydrate (molasses, mealies) give a certain protection against prussic acid. It is also said that lucerne hay and linseed cake retard the production of prussic acid and in this way may prevent poisoning (Couch, 1932).

Investigations made by Steyn (1931A) have shown that sulphur is an excellent preventive of "geilsiekte" (prussic acid poisoning due to the ingestion of certain wilted grasses) in sheep.

Farmers are advised to combat the disease by mixing sulphur with their sheep licks. On farms deficient in minerals, i.e. where the animals eat bones, rags, etc., the lick should not contain more than 5 per cent. sulphur; and on farms where the mineral shortage

is not so pronounced, the lick may contain 7½ per cent. sulphur (1 part sulphur to 13 parts of any lick which does not already contain sulphur). Copper sulphate (blue stone), Cooper's Dip and the Government wireworm remedy were also tested, but the best results were obtained by using sulphur.

When climatic conditions are favourable for "geilsiekte", that is, light rains after prolonged droughts, with spells of sunny weather, or, during an actual outbreak of "geilsiekte", every sheep or goat above the age of six months may be given a well-filled teaspoon of sulphur ($= \pm 5 \cdot 0$ gm.) every fourth day, while the animals should at the same time have free access to the sulphur lick. Sheep and goats under the age of six months should be given half a teaspoon of sulphur. Calves not receiving sulphur licks may receive the same dose of sulphur as full-grown sheep and full-grown bovines up to two tablespoons of sulphur ($= 30$ gm.) daily over unlimited periods (Onderstepoort experiments conducted by the author). The animals should not be dosed continuously for longer than one month in addition to having access to sulphur licks.

II. ALOPECIA (KAALSIEKTE) IN KIDS AND LAMBS CAUSED BY PLANT POISONING.

(*Chrysocoma tenuifolia* Berg.)
(O.P. No. AW; 1929. N.H. No. 14407.)

Introduction.

Kaalsiekte (*alopecia*), which literally means "naked-disease", is the term applied to a disease in lambs of cross-breeds of sheep and in kids, the most outstanding symptom of which is partial or complete loss of the coat. Hence the appropriate name assigned by farmers to this disease.

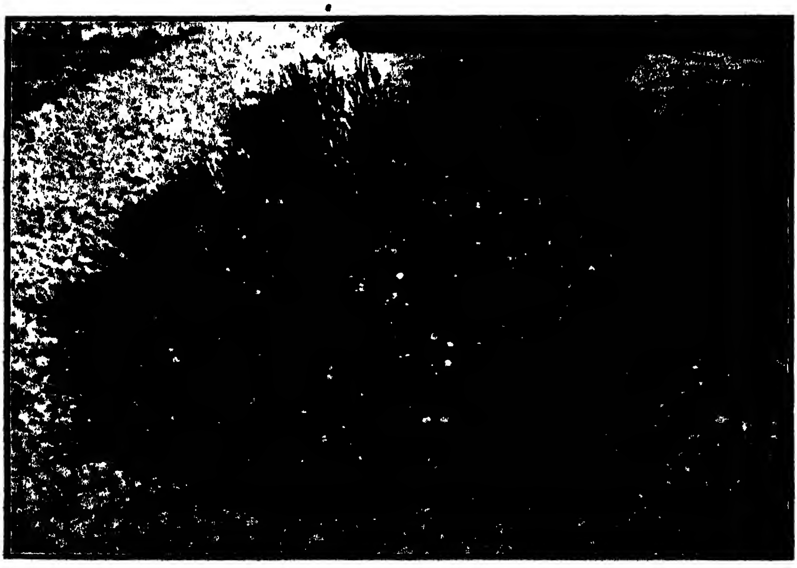

Fig. 1.—*Chrysocoma tenuifolia* Berg. (Plant used in feeding experiments.)

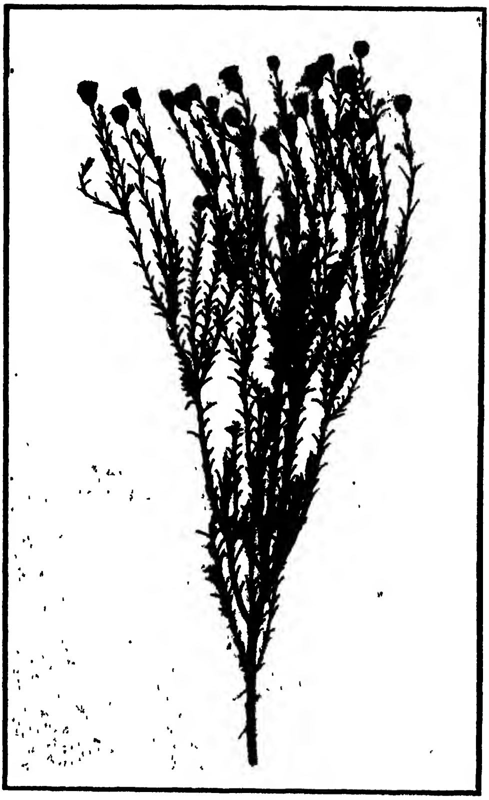

Fig. 2.—*Chrysocoma tenuifolia* Berg. (Plant used in feeding experiments.)

Alopecia, although it had existed in the Willowmore Uniondale and other neighbouring districts for the last seventy or eighty years, according to reliable information supplied by elderly and experienced farmers, was for the first time investigated and reported upon by Van Rensburg (1925), the then lecturer in veterinary science at the Grootfontein School of Agriculture, Middelburg, Cape Province. Specimens of the skin and thyroid glands of affected kids were forwarded to Onderstepoort for histological examination. The skin showed a crystaceous eczema and the thyroids revealed no specific changes.

As the affected area in the Willowmore and Uniondale districts alone carries approximately two hundred and fifty thousand Angora and mixed breed of goats, it was decided to have this disease investigated.

Incidence and Statistics.

The disease is said to be limited to " sour veld ". A sheep inspector who has had many years' of experience as sheep inspector in the areas where alopecia is prevalent, has informed the author

that he has also seen the disease in the Cradock, Hofmeyr, Tarkastad, Middelburg (Cape), Colesberg, Graaff-Reinet, Pearston and Jansenville districts. In these districts, he continued, the disease invariably appears on farms with deficient soil which produces very little, if any, edible vegetation of a high nutritive value. In the course of his investigations the author had ample opportunity of verifying the latter statement.

Mr. O. T. de Villiers, Government Veterinary Officer, Middelburg, Cape, who investigated the disease in the Cradock district, reported that it is most prevalent in the mountainous parts of that district. This has been the author's experience in the Willowmore and Uniondale districts, where the disease is limted to the north-western part of Uniondale and the south-eastern part of Willowmore.

It is impossible to express in exact figures the losses caused by this disease. These are, however, enormous, as can be gauged by the fact that in the Willowmore and Uniondale districts alone over two hundred thousand susceptible small stock are running in the affected areas. On many farms it is impossible to rear a single kid, or lamb of mixed breeds of sheep. On such farms it is a general practice not to attempt to rear any kids, or lambs of cross-breeds of sheep, but to buy three to six months old kids and lambs, which then mature on these farms without any apparent ill-effects.

In addition to the losses amongst the young of the small stock the owner of an "alopecia farm" suffers a direct heavy financial loss due to the fact that such a farm loses considerably in value as far as the grazing capacity for small stock is concerned.

Time of the Year.

The severity of the disease varies considerably from year to year. In years when late winter or early summer rains fall the disease is much more prevalent than in dry years, during which the disease either does not appear or occurs in a very mild form, even on the most notorious alopecia farms. The disease is most prevalent during the period August to October, although severe outbreaks have been known to occur during May, June and July. The writer has seen the disease in June, 1930, in the Willowmore district.

Species of Animals Affected.

Cases have appeared in the lambs of cross-breeds of all sheep, in Angora kids and in the kids of cross-breeds of goats. The disease exhibits itself in from four to fourteen days old lambs and kids and rarely makes its appearance in these animals after the age of two weeks. During the worst outbreaks cases have been reported to occur in kids up to one month old. The affected areas carry the above breeds of small stock but no Merino sheep. None of the many farmers interviewed could supply the author with any information regarding the occurrence of the disease in Merino lambs.* Donkeys, horses and cattle also run in the alopecia areas, but no cases of this disease have ever been known to appear in the young of these animals.

* Van Rensburg (1925), however, mentions that some farmers reported that they have seen alopecia in Merino lambs.

Morbidity and Mortality.

The morbidity depends to a considerable extent on the rainfall. It is highest after late winter or early summer rains, when the morbidity might be as high as 100 per cent. We must bear in mind the fact that the kidding season in the affected areas extends usually from the beginning of August to the end of October.

The mortality depends largely on the care given to the affected animals. In cases of negligence the mortality may be as high as 90 per cent., whereas it could be reduced to 50 per cent. or less with proper treatment. This point will be discussed under " Treatment ".

Symptomatology.

The symptoms can best be divided into primary and secondary.

Primary Symptoms.—These are the shedding of the coat and diarrhœa. The former symptom appears in about 95 per cent. of the cases and frequently is associated with diarrhœa. It rarely happens that the affected kids and lambs develop a pronounced diarrhœa and die before loss of hair occurs.

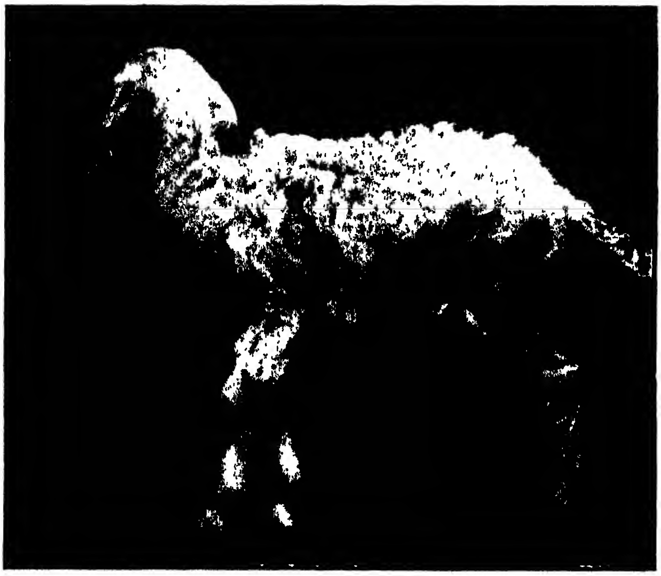

Fig. 3.—Alopecia. Note loss of hair on side of body. Experimental case.

On careful observation the first discernible symptom is itching and the affected animals can be seen scratching and biting their sides. At this early stage the hair, especially over the sides, can be easily removed by hand. Macroscopically the skin on the affected parts of the body appears normal. In bad cases such animals lose practically the whole coat overnight and it continually seeks shelter. In mild cases the coat is shed over a period of a few days provided the affected animal survives the diarrhœa.

Invariably the first bald patches are to be seen over the shoulder blades and the upper half of the hind legs (see Fig. III). By pulling the hair on these parts an affected case can be detected in its earliest stages. Many cases in the initial stages can be picked out by the peculiar ruffled appearance of the hair over these parts.

As soon as diarrhœa sets in the animals show inappetence, listlessness and, frequently, fever. In cases of severe diarrhœa the animals die in one to three days without loss of hair.

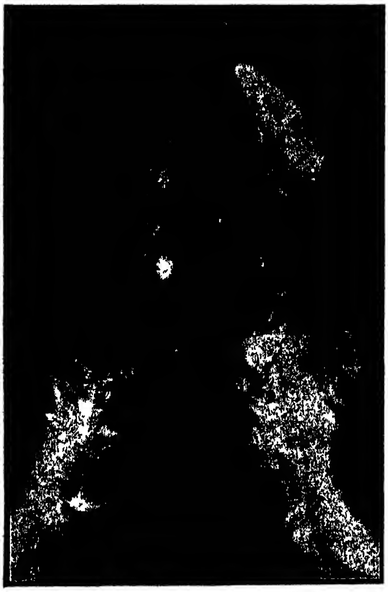

Fig. 4.—Alopecia. Kid showing profuse diarrhœa and hairless patches on hind legs. Experimental case.

In the course of a few hours to a few days, depending on the severity of the case, the affected kids and lambs become completely bald (see Fig. II), with a little hair left on the lower parts of the legs, the tips of the ears and tail, and on the head. These animals present a most peculiar appearance. In this state the animals show symptoms of a marked general disturbance, e.g. fever, diarrhœa, inappetence, marked depression, staggering gait and, ultimately, inability to rise.

The hair on the coloured patches of the skin (in mixed breeds of sheep and goats) is much more resistant to the effects of the toxin than that on the unpigmented parts, with the result that the coloured patches are still covered by hair, while the unpigmented parts of the skin are completely bald. Likewise, black and brown animals are more resistant to alopecia. However, in bad cases these animals also lose their hair, provided they do not die within a short time from diarrhœa.

In addition, the affected kids develop an acute conjunctivitis, keratitis and rhinitis (see Fig. IV). Permanent loss of eyesight is, however, very rare.

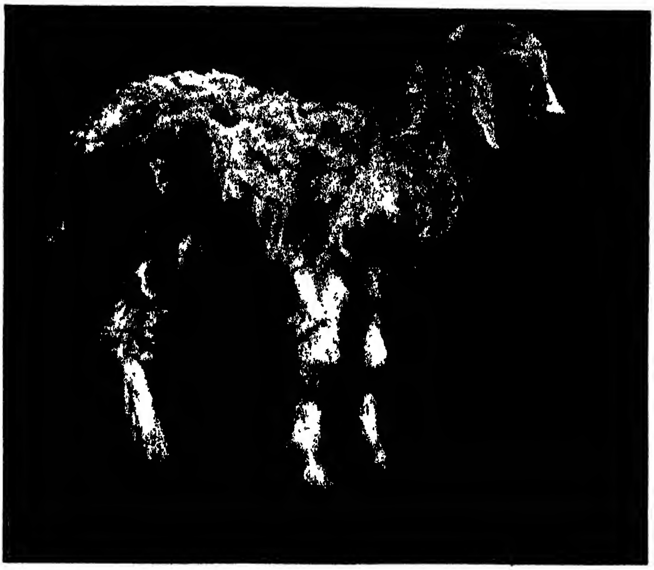

Fig. 5.—Alopecia. Condition more advanced. Diarrhœa and acute purulent conjunctivitis and rhinitis present. Experimental case.

Fig. 6.—Alopecia. Dermatitis setting in. Note the bad condition of the animal. Experimental case.

Fig. 7.—Natural case of alopecia.

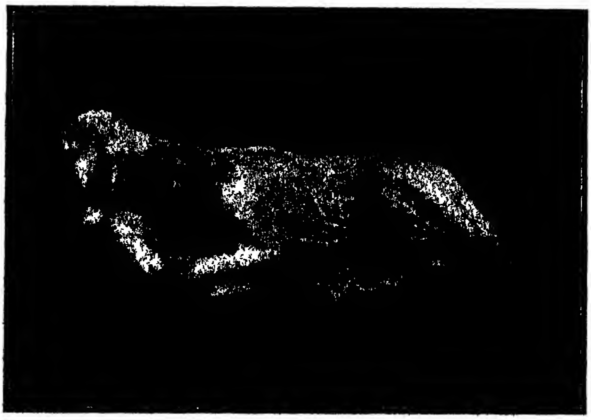

Fig. 8.—Natural case of alopecia showing almost complete hairlessness. The skin appears quite normal.

Fig. 9.—Natural case of alopecia. Acute crustaceous dermatitis.

Fig. 10.—A kid recovering from alopecia. A new coat is appearing. Natural case.

Secondary Symptoms.—These are an acute dermatitis, pronounced diarrhœa, obstruction of the gastro-intestinal tract by hair-balls, and acute catarrhal pneumonia.

The acute dermatitis is caused most probably by the action of sunlight on the unprotected skin (see Fig. VII). It commences on the hairless patches with an intense reddening, swelling, painfulness and, later on, exudation. In the course, of time, hard crusts are formed on the skin. It is evident from Fig. IV that such animals experience severe pain when walking or standing with straightened legs and back, as this causes stretching of the skin.

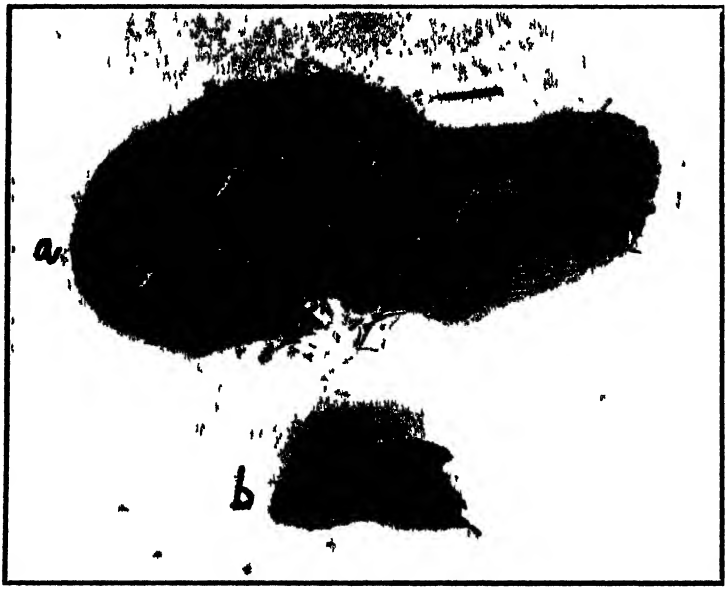

Fig 11.—(*a*) Ruminal and (*b*) abomasal hairballs collected from a kid which had died from alopecia.

Diarrhœa almost invariably accompanies the process of shedding of the hair. Diarrhœa in alopecia in kids and lambs may be caused either primarily by the toxin, as it is present in cases where no loss of hair occurs, or secondarily, by the irritation produced by the ingestion of large amounts of hair, or by both these factors. Affected kids can be seen pulling out mouthfuls of hair from the itching skin, and chewing and swallowing them.

It rarely happens that kids shed the coat and die within a comparatively short time without developing a diarrhoea. In these cases autopsy reveals one or more hairballs completely obstructing the pyloric portion of the abomasum (see Fig. IX). The abomasum is distended by intense malodorous gases and its contents are in a state of advanced decomposition. Many farmers have informed the author that they have lost kids from the effects of hairballs up to three months after these animals had recovered from alopecia.

In cold weather a large percentage of the hairless kids develop a fatal acute catarrhal pneumonia if the necessary provisions for shelter are not made.

Post-mortem Appearances.

As far as the external lesions are concerned these coincide with those described under Primary and Secondary Symptoms. An acute catarrhal gastro-enteritis is present in cases which have exhibited a diarrhoea. In all cases where alopecia is present autopsy reveals an enormous amount of hair mixed with the gastro-intestinal contents, and hairballs varying in size. As has been described before, the latter might cause death before the gastro-intestinal irritation has been produced.

An acute catarrhal pneumonia frequently is the cause of death in hairless kids and lambs and may be complicated by one or more of the other secondary conditions.

Nature and Cause of the Disease.

This aspect of the disease will be discussed under " Experiments to determine the Cause of Alopecia in Kids and Lambs ".

Treatment.

The careful nursing of the affected animals is of much greater importance than the treatment which is not of much use. Treatment can be carried out on general principles.

Mild oily laxatives, for example, raw linseed oil, are indicated to remove the hair and hairballs from the gastro-intestinal tract. This treatment must be commenced as soon as the kids are noticed to bite and scratch, as in the course of a day or two the hairballs reach such dimensions as will render their escape from the abomasum into the small intestine impossible.

In diarrhoea small quantities of raw linseed oil and limewater prove beneficial.

In pneumonia treatment generally is of no avail, it could be carried out on general principles.

The dermatitis can be treated with liniments to prevent the skin from hardening and to allay the irritation, and farmers have reported very favourably on the effects of lanolin on the bald and burnt skin.

Prevention.

Prevention as far as the primary symptoms are concerned will be discussed in the second part of this paper.

Many farmers although experiencing a high percentage of alopecia amongst their animals, have reduced their losses to a minimum by erecting a small shed at the kraal where the kids and lambs are kept. These sheds afford the necessary shelter against cold and the burning rays of the sun, with the result that pneumonia and dermatitis rarely develop.

Experiments to Determine the Cause of Alopecia in Kids and Lambs.

A.—Experiments on the Farm Skilpadbeen, Willowmore.

Preliminary Investigations.

The author investigated the disease on a number of farms in those parts of the Uniondale and Willowmore districts where the disease is most prevalent. In addition, alopecia-free farms were visited for purposes of comparison.

First of all the nature of the disease had to be settled. Was it a deficiency disease or infectious disease or a plant intoxication?

The following facts gathered in the course of the investigation pointed strongly to plant poisoning:—

(*a*) The disease does not appear when the pregnant goats and cross-breeds of sheep are turned on to green barley or oat-lands fourteen days before kidding and lambing commence and kept there until fourteen days after kidding and lambing. The same applies when the pregnant goats and sheep are allowed to graze high up in the mountains for the above period. The disease is also known to be more severe on certain parts of one and the same farm than on other parts.

(*b*) The disease is most prevalent in years when the rainfall is at its highest. At such times there is a luxuriant growth of herbage and we must consider that certain plants may be eaten only when in the flowering stage. In addition, in good years the milk yield is much higher than in years of drought. Farmers have informed the author that in years of drought the morbidity may be 1 per cent., or even less, whereas on the same farm the morbidity might be as high as 100 per cent. during years with a high rainfall.

(*c*) The disease can be controlled to a certain extent by partially emptying the mother's udder before the young ones suck. This method of preventing the disease is practised by many farmers with a fair amount of success. The less milk the kids get, the less " poison " they ingest.

(*d*) Twins and triplets are much less susceptible to the disease than in cases where only one kid or lamb sucks the mother. This again is a case of the young receiving less milk and consequently less " poison ".

(*e*) No new outbreaks are experienced as soon as the kids and lambs are weaned.

(*f*) Kids and lambs are never born bald, the earliest cases of alopecia appearing in three days old kids and lambs. It is most prevalent in kids and lambs from four to fourteen days old, less prevalent in three weeks old kids and lambs, and is rarely seen after these animals have passed the age of three weeks.

(*g*) It is common experience that kids or lambs sucking a mother-goat or sheep, whose young have died from the effects of alopecia, will also contract the disease.

It has been suggested that alopecia is due to a deficiency of iodine. The above-mentioned points, as well as the fact that the kids and lambs are born in a state of perfect health with a perfectly normal coat, speak against this theory. In the face of the above facts it is unnecessary to elaborate on the symptoms of iodine deficiency.

It is of interest to mention the views expressed by the farmers as to the cause of the disease. These can be summed up as follows:—

(*a*) A number of farmers ascribed the disease to the mother-goats and sheep eating the so-called "opslag" after the rains. The term "opslag", as used by the farmer, may include any plant whose growth is dependent on the late winter or early summer rains.

(*b*) The following plants have been incriminated: The geelbos (*Lopholaena Randtii*); the beesbossie, also known as the bitterkarroo, brandbossie; bitterbossie (*Chrysocoma tenuifolia*); Euphorbia species; and a number of other plants. Some farmers maintained that the mothers had to ingest more than one plant at the same time in order to cause the development of alopecia in their young.

(*c*) Many believed the water to be the source of the trouble.

(*d*) Others again were baffled by the disease and could express no opinion as to its possible cause.

(*e*) Many farmers regarded Alopecia as a deficiency disease and claimed good results by supplying the mothers with licks containing iron sulphate and bonemeal.

(*f*) Some farmers maintain that they combat the disease very successfully by feeding a salt-bonemeal lick to the breeding stock.

The results of investigations made by the author pointed to alopecia in kids and lambs being due to plant poisoning, the pathogenesis of which is as follows: The mother of the affected kids or lambs ingests a plant, which apparently causes no harmful effects on the full-grown animals, but whose active principle is secreted in the milk and in this way produces the so-called alopecia in the kids and lambs.

The water as a possible source of the trouble could be eliminated, as the disease could be prevented by allowing the mothers to graze on green oats or barley lands, while the water supply remained the same.

A careful botanical survey of a number of affected and unaffected farms was then made. The unaffected farms and the "alopecia-free" and "alopecia" portions of the affected farms were carefully

examined with the result that the number of suspicious plants were reduced to two, namely, the beesbossie *(Chrysocoma tenuifolia)* and the Bothablom *(Polygala teretifolia)*.

It was then decided to conduct experiments with these plants.

Arrangements were accordingly made with Mr. M. J. Ferreira, Skildpadbeen, Willowmore, to conduct the experiments on his farm, which in the past has been so bad with the disease that the owner had practically stopped breeding kids and lambs, and resorted to the method of buying three to six months' old kids and lambs for purposes of speculation.

Field Experiments in the Willowmore District.

The undermentioned experiments with highly pregnant Angora goats were commenced on 12th August, 1930, approximately two weeks before kidding was due.

Experiment I.

To determine whether *Chrysocoma tenuifolia* Berg. is the cause of alopecia in kids.

Common names of plants: Bitterkarroo, beeskarroo, bitterbossie, brandbossie.

Fifteen goats were fed as follows:—

(*a*) Bitterkarroo only: 5 goats.

(*b*) Bitterkarroo + 0·1 gram potassium iodide per head daily: 5 goats.

(*c*) Bitterkarroo + two tablespoons of salt-bonemeal lick (1 : 2) daily: 5 goats.

These animals were placed in a specially constructed wire-netting kraal twenty-six by sixteen yards, from which all the vegetation, except the bitterkarroo, was removed. The animals immediately commenced eating the bush and completely cleared the kraal in six hours. From 13.8.30 the animals received approximately sixty pounds of the freshly cut bush per day. The flowering plant only was utilized in the feeding experiments and was collected by cutting off the upper four inches of the bush. The material, which was scattered in a shady place, in order to prevent rapid wilting, was very eagerly eaten by the goats.

All the experimental goats received their drinking water from the same source as the flock in order to ascertain whether the drinking water played any part in the causation of the disease.

The five goats in group (*b*) were dosed daily with 0·1 gram potassium iodide in order to exclude an iodide deficiency. The potassium iodide was dissolved in ordinary spring water and dosed by means of a syringe.

Each of the five goats in group (*c*) received daily two tablespoonfuls of a mixture of one part of salt and two parts of bonemeal, in order to determine whether these substances had any direct effect on the disease, preventively or curatively. It should be mentioned that many farmers in the "krimpsiekte" areas of the Uniondale and Willowmore districts, where this disease

is caused by *Cotyledon ventricosa* and *Cotyledon wallichii* informed the author that they have reduced their losses from "krimpsiekte" to a negligible number by allowing their stock free access to a salt-bonemeal lick.

It was the intention to include an additional experiment by daily force-feeding twenty pregnant goats with the salt-bonemeal lick and allowing them to run with the flock, which had to serve as controls. This experiment was abandoned, as Mr. Ferreira allows all his animals free access to a salt-bonemeal lick.

On the fourth day after the commencement of the experiment, that is, after each animal had ingested approximately sixteen pounds of the flowering tops of the frshly cut plant, all the animals showed a marked diarrhœa, which in the course of a few days became very acute. The animals exhibited pronounced straining, evacuating varying quantities of a very fluid greenish material mixed with large amounts of mucus which from a few animals was mixed with blood. There was extremely rapid loss in condition, and the animals drank enormous amounts of water. The eyes were sunken and the nostrils showed a dirty mucous discharge.

The visible mucous membranes were extremely pale and there was complete absence of appetite. Furthermore, the animals exhibited a general weakness, which progressed until they were unable to rise. The respiration was very rapid, costo-abdominal and shallow. The pulse was accelerated and in the course of time became very irregular and ultimately imperceptible. In the last stage of the disease the animals lay on their sides utterly exhausted and helpless until death intervened.

Four of the fifteen animals aborted in the course of the disease, and it is of interest to note that three of these animals were receiving 0·1 gram potassium iodide daily.

Three animals died and one was killed *in extremis* sixteen days after commencement of the experiment.

Post-mortem Appearances.

Anaemia, cachexia, hyperaemia of the lungs and liver; heart extremely flabby and both ventricles markedly distended with coagulated blood; acute catarrhal gastro-enteritis. The four animals showed advanced pregnancy.

All the affected animals were treated with a mixture of limewater and raw linseed oil with very unsatisfactory results. As mentioned above, four died and the rest recovered extremely slowly.

Only seven pregnant animals were left, and of these one had to be discharged on account of its bad condition. Another nine available pregnant Angora goats were placed in the experiment in order to bring the number up to fifteen and the experiment continued from 1.9.30.

During field observations it was noticed that both sheep and goats fed extensively on the flowering bitteıkarroo without any ill-effects. It was thought that symptoms of bitterkarroo poisoning could be prevented when additional food was given to the experimental animals. Accordingly from 1.9.30 the fifteen experimental animals received approximately sixty pounds of the freshly cut flowering bitterkarroo in the morning, and as soon as this had been ingested they were offered twenty pounds of lucerne-hay and forty pounds of green barley. Of the lucerne-hay and barley they took variable quantities, whereas of the bitterkarroo each animal daily took approximately four pounds of the freshly collected flowering plant. In spite of the fact that the animals were daily fed as above from 1.9.30 to 27.9.30, they developed no symptoms of ill-health.

Eleven out of thirteen kids born from the above goats developed typical symptoms of alopecia as it occurs under natural conditions. Of the affected kids eight died and one was killed *in extremis*.

The results of this experiment are summarized in the following table:—

TABLE 1.

Chrysocoma tenuifolia fed to Pregnant Goats.

No. of pregnant goats.	Material received.	Date of birth of kids.	Date on which symptoms of alopecia appeared in kids.	Interval between date of birth and date of appearance of symptoms.	Remarks.
1	Bitterkarroo only	—	—	—	Died on 27/8/30 from effects of bitterkarroo.
2		—	—	—	Died on 26/8/30, from effects of bitterkarroo.
3		Aborted on 27/8/30	—	—	Discharged.
4		—	—	—	Killed *in extremis* on 28/8/30. Condition due to ingestion of bitter-karroo.
5		9/9/30	18/9/30	9 days	Alopecia diarrhœa. Died on 1/10/30.
6		11/9/30	14/9/30	3 days	Slight alopecia. No diarrhœa. Died on 14/9/30
7		16/9/30	23/9/30	7 days	Alopecia and diarrhœa. Died on 1/10/30.
8		16/9/30	27/9/30	11 days	Alopecia and diarrhœa. Died on 29/9/30.
9		19/9/30	24/9/30	5 days	Alopecia and diarrhœa. Died on 30/9/30.
1	Bitterkarroo + potassium iodide	Aborted on 19/8/30	—	—	Discharged.
2		Aborted on 20/8/30	—	—	Discharged.
3		Aborted on 23/8/30	—	—	Discharged.
4		7/9/30	—	—	No alopecia developed.
5		9/9/30	—	—	Mother had no milk, hence discharged.
6		16/9/30	22/9/30	6 days	Alopecia + diarrhœa. Died on 26/9/30.
7		Aborted on 16/9/30	—	—	Discharged.
8		Aborted on 16/9/30	—	—	Discharged.
1	Bitterkarroo + salt-bone-meal lick	18/8/30	—	—	Kid died on 22/8/30. Cause of death unknown Mother discharged.
2		—	—	—	Died on 27/8/30 from effects of bitterkarroo.
3		8/9/30	18/9/30	10 days	Alopecia + diarrhœa. Died on 30/9/30.
4		12/9/30	19/9/30	7 days	Alopecia. Died on 26/9/30.
5		16/9/30	27/9/30	11 days	One kid developed alopecia and recovered, whereas other one remained healthy.
6		19/9/30	23/9/30	4 days	Alopecia and diarrhœa. Killed *in extremis* on 27/9/30.
7		19/9/30	27/9/30	8 days	Alopecia + diarrhœa. Recovered.

Discussion.

From the above table it is evident that—

(*a*) five goats of the potassium iodide group aborted, and one of the bitterkarroo group;

(*b*) eleven out of thirteen experimental kids developed typical symptoms of alopecia, and of these eight died and one was killed *in extremis;*

(*c*) only one of the twin kids developed alopecia;

(*d*) the earliest case of alopecia appeared three days and the latest one eleven days after birth;

(*e*) death occurred two to twelve days after the appearance of symptoms; one kid died on the day alopecia was noticed;

(*f*) three pregnant goats died from the effects of the bitterkarroo and one was killed *in extremis.*

The appearance, course, symptoms and post-mortem appearances of alopecia, as it was noticed in the experimental kids, coincided completely with those encountered in the natural cases of the disease.

The mother goats, although well-fed, were much worse in condition and consumed much larger quantities of water than the fifteen animals in Experiment II, which, in addition to the "Bothablombos", received the same amount of lucerne-hay and green barley as the goats in Experiment I.

EXPERIMENT II.

Polygala teretifolia, Thunb.

Common name: Bothablombos.

Fifteen pregnant Angora goats were placed in a pen erected about fifty yards away from that of Experiment I and received daily sixty pounds of the fresh flowering tops of the "Bothablombos" from 12.8.30 to 27.9.30. The animals picked off all the flowers and took very little of the leaves of the plant, with the result that they ingested only about thirty of the sixty pounds of plant offered them. As the animals steadily lost in condition they received twenty pounds of lucerne-hay and forty pounds of green barley in the afternoon.

The following experiments were conducted:—

(*a*) Bothablombos only: 5 goats.

(*b*) Bothablombos + 0·1 gram potassium iodide per head daily: 5 goats.

(*c*) Bothablombos + two tablespoonfuls of salt-bonemeal lick (1 : 2) per head daily: 5 goats.

Up to 1.9.30 two goats in (*b*), one in (*c*), and one in (*a*) aborted. These four animals were replaced by four pregnant goats on 1.9.30. On 5.9.30 another potassium iodide goat aborted. The rest of the goats gave birth to normal kids. At the time the experiment was discontinued there were ten kids ranging in age from two to five weeks. Both the kids and their mothers were in good condition and perfect health.

Discussion.

The fresh "Bothablombos" in the quantities fed had no deleterious effects on the experimental animals. Three potassium iodide goats, one salt-bonemeal goat, and one "Bothablombos" goat aborted.

EXPERIMENT III.

Twenty pregnant Angora goats, which daily received 0·1 gram potassium iodide from 12.8.30, were allowed to run with the flock. As a high percentage of abortions occurred in the animals receiving the potassium iodide, the quantity was reduced to 0·05 gram daily from 1.9.30. From this date onwards all the experimental animals in the potassium iodide groups received 0·05 grams per head.

Up to 14.9.30 five of the twenty goats aborted. At the time the experiment was discontinued, the fifteen remaining goats had kids ranging in age from eleven days to six weeks. Amongst these kids only one very light case of alopecia occurred.

Experiment IV.

Twenty pregnant Angora goats, each of which received daily 5 grams of sulphur, were allowed to run with the remainder of the flock.

On 27.9.30, when the experiment was discontinued, the kids ranged in age from twelve days to six weeks. Only one case of alopecia appeared amongst these kids. It is of interest to note that no abortions occurred in this group of animals.

Controls.

The rest of the flock of pregnant goats, numbering about three hundred, which had free access to a salt-bonemeal lick (1 : 2), was kept as controls.

As the owner was advised to keep the pregnant animals away from the "bitterkarroo" veld, the flock of pregnant goats were allowed to graze high up in the mountains during daytime, and only had the opportunity of feeding on the bitterkarroo, in the evening when they were brought home, and early in the morning when they were driven out.

This accounts for the extremely low percentage, namely, 2·4 per cent., of alopecia which occurred in the flock, of which two hundred and fifty had already kidded at the time the experiment was discontinued. Of these animals six had aborted, and amongst the kids only six cases of alopecia appeared.

The owner stated that in good years, such as 1930, up to 90 per cent., of his kids developed alopecia.

Cotyledon wallichii and *Cotyledon ventricosa* must be considered as a possible cause of the abortions in the control flock, as these two plants, especially the latter, grow luxuriantly on the mountain slopes. The author witnessed a number of cases of "krimpsiekte" in the control flock, and one goat had actually aborted twins during an attack of "krimpsiekte".

Experiments Conducted at Onderstepoort.

Since it was established that *Chrysocoma tenuifolia* (bitterkarroo) is the cause of alopecia, which occurs so extensively in kids and lambs of mixed breeds in the Willowmore and neighbouring districts, it was decided to continue the investigations at Onderstepoort. The first point that had to be settled was whether the plant in the dry state was capable of producing the disease. This naturally was of the utmost importance, as it was the most important factor to determine whether or not it would be possible to conduct experiments at Ondersteporto. As will be seen in the course of the undermentioned experiments, the dried plant was found to produce the disease, and it was proposed to investigate the following points in connection with alopecia : :—

(*a*) The susceptibility of Merino lambs to alopecia.

(*b*) The age factor. This is a point of enormous practical and economical importance. How long before kidding must the pregnant goats and sheep be removed from the "bitterkarroo" veld in order to prevent alopecia? Experienced farmers maintain that no cases of alopecia will appear if the pregnant animals are removed from "alopecia veld" fourteen days before kidding and kept away from such veld until the kids are fourteen days old. This "time limit" could be easily determined by experiment. It will be realized that this "time limit" is of the utmost importance, as the feeding of thousands of pregnant animals involves large sums of money and frequently is impossible. It is, therefore, in the interest of the farmers concerned to know exactly how long to keep their animals away from "alopecia veld".

(*c*) Is the alopecia-toxin prepared in the system or is it contained as such in the plant?

(*d*) Does the " bitterkarroo " from " alopecia-free " areas also produce the disease? It is an interesting fact that alopecia only occurs in certain parts of the affected districts. In the Willowmore and Uniondale districts the disease is very prevalent in the mountainous parts, whereas it is of extreme rare occurrence on the even veld. The grazing on the " alopecia farms " is very poor, with the result that the animals have to rely to a large extent on the " bitterkarroo " for their food, whereas the veld of the " alopecia-free " farms is of a much superior quality. Is the quality of the veld or a difference in the toxicity of the responsible plant in different localities the determining factor in the occurrence of alopecia?

PRELIMINARY EXPERIMENTS.

Experiment 1.

To ascertain whether the plant in the dry state will still produce alopecia.

Pregnant Angora goats obtained from the Willowmore districts were employed in these experiments. The " bitterkarroo " bush was collected in the flowering stage on the farm Skildpadbeen, and sun-dried and forwarded to Onderstepoort.

Goats 29207 and 29214 were starved for twenty-four hours and then offered the dried " bitterkarroo ". As nothing was ingested during the following twenty-four hours, the dried bush was cut up coarsely and mixed with lucerne-hay. During the following three days the animals did not ouch the mixture. As a good milk-yield is essential for the production of alopecia, it was thought inadvisable to discontinue starvation, and drenching was resorted to in all the following experiments.

The above animals received daily (except Sundays) 400 grams of the dry plant from 27.10.30. On 8.11.30 No. 22907 aborted and was discharged. As the abortion was most probably caused by the " bitterkarroo ", this was an indication that the animals were receiving too large quantities of the plant. Consequently goat 29215, together with goats 2906 and 29203, the latter two having been added to the experiment on 10.11.30, received daily (except Sundays), 200 grams of the plant until the date of lambing, when the dose was increased to 400 and 800 grams on alternate days.

Result.

Goat 29203.—This animal kidded on 30.11.30 (kid 29371). On 16.12.30 it accidently inspired some of the drenching material with fatal results. Up to the time of death this animal had received 12·4 kilograms of the dried plant in the course of five weeks without any deleterious effects.

Kid 29371 (born on 30.11.30).—9.12.30: Diarrhœa. 10.12.30: Diarrhœa with straining. At 2 p.m. the animal was noticed stamping with the hindlegs, swishing the tail, running about and biting at its sides and hindlegs. It was seen chewing and swallowing the hair which had been pulled out during the biting. On closer examination it was found that the hair over the shoulder-blade, the sides and the lateral aspects of the hindlegs could be removed very easily (see Fig. I). Temperature: 103·4° F. The animals keenly sought shade. Macroscopically the skin appeared perfectly normal.

11.12.30.—Diarrhœa. Sides almost completely hairless; hair on neck and back easily removable. Temperature: 103·8° F. Pronounced acute catarrhal conjunctivitis. Losing in condition. Pulse: 120, strong. Inappetence.

12.12.30.—Diarrhœa pronounced. Hairless areas on the skin on the sides are red, warm, painful, and swollen. Temperature: 103·6° F. Pulse: 124, strong. Inappetence.

13.12.30.—Diarrhœa pronounced. Losing in condition. Shedding of the coat continued. Acute catarrhal conjunctivitis. The hairless portion of the skin shows an acute dermatiis. Animal walking with stiff legs, as movement causes stretching of the inflamed skin with consequent pain. Temperature: 104·4° F. Animal shows symptoms of severe irritation of the skin. Pulse: 118, strong. Inappetence.

14.12.30, 15.12.30, 16.12.30.—Condition as on 13.12.30. As the mother goat 29203 died, on 16.12.30, the kid was handreared from this date onwards on cow's milk. Inappetence.

17.12.30.—Diarrhœa pronounced. Losing in condition and very apathetic. Moist dermatitis. Still shedding coat. A bilateral purulent conjunctivitis with the lids of the eyes glued together. An increased discharged from the nostrils. Temperature: 103·6° F. The inflamed skin over the lateral aspect of the thigh shows a dry crustaceous dermatitis with deep blood-stained cracks (see Fig. III). Pulse: 132, strong. Inappetence.

18.12.30.—Diarrhœa pronounced. Condition bad. Hairless portion of skin swollen, reddened, painful, and hard. Animal appears hidebound, stands with back arched and moves with difficulty. Temperature: 103·4 F. Pulse: 128, strong. Inappetence.

19.12.30, 20.12.30.—Condition as on 18.12.30. Animal almost hairless. Little hair left on the legs, back, and head. Diffuse crustaceous dermatitis. Inappetence.

21.12.30.—As on 20.12.30.

22.12.30.—New hair appearing on the first hairless patches. Diarrhœa pronounced, acute purulent conjunctivitis and rhinitis. Diffuse crustaceous dermatitis, with bleeding cracks. Temperature: 102·8° F. Not feeding. Condition very bad. Pulse: 124, strong.

The above-described skin lesions and symptoms culminated in death during the night of 26.12.30.

Post-mortem Appearances.—Almost complete hairlessness with new coat appearing on some parts of the skin; cachexia, anaemia; acute purulent conjunctivitis and rhinitis; acute crustaceous dermatitis, hyperaemia of the lungs and liver; marked atrophy of the spleen; hairballs in rumen [see Fig. IX (*a*)] and abomasum [see Fig. IX (*b*)], the latter completely obstructing the pyloric portion of the abomasum; acute catarrhall duodenitis, jejunitis and colitis; entire gastro-intestinal tract completely devoid of ingesta.

Goat 29214.—This animal gave birth to a normal kid (No. 29373) on 5.12.30. From 27.10.30 to 5.13.30 goat 29214 received 23·2 kilograms of the dry plant without developing any symptoms of ill-health.

Kid 29373 (born 5.12.30). 11.12.30.—Stamping with hind feet, swishing the tail, running about, and biting at external surface of hind legs. On closer examination the hair on the sides and external surfaces of the legs and to a slighter extent on the back can be easily removed. Temperature: 103·8° F. Pulse and respiration normal. No diarrhœa.

12.12.30.—Hairless patches on the sides and back. Temperature: 103·9° F. Animal exhibits symptoms of marked irritation of the skin. Biting at the sides, chewing and swallowing the extracted hair.

13.12.30.—As on 12.12.30.

14.12.30.—Sides and lateral aspect of the thighs completely hairless. The hairless portions of the skin show signs of inflammation.

15.12.30.—Diarrhœa. Shedding of coat progressing. Temperature: 103·4° F. Slight catarrhal conjunctivitis.

16.12.30.—Pronounced diarrhœa. Losing in condition. Alopecia progressing. Acute catarrhal conjunctivitis and rhinitis. Temperature: 103·4° F. Pulse: 118, strong.

17.12.30, 18.12.30, 19.12.30, 20.12.30.—Condition growing worse. Pronounced diarrhœa and loss in condition. Acute purulent conjunctivitis and rhinitis. Shedding of coat and dermatitis progressing. Temperature: 102·8° F. Pulse: 124, strong.

22.12.30.—Pronounced diarrhœa. New hair appearing on the hairless portion of the skin. From this date onwards there was steady improvement in the condition of the animal until complete recovery on 5.1.31.

TABLE II (Experiment I).

Dried Willowmore "Bitterkarroo".

Goat No.	Date on which dosage commenced.	Period of dosage.	Quantity of dry plant received.	Kid No.	Date of birth.	Date of appearance of alopecia symptoms.	Remarks.
29207	27/10/30	11 days	4 kg.	—	—	—	Aborted on 8/11/30.
29203	10/11/30	35 days	12·4 kg.	29371	30/11/30	9/12/30	Diarrhœa appeared on 9/12/30, and alopecia on 10/12/30. Kid died on 26/12/30.
29214	27/10/30	29 days	23 2 kg.	29373	5/12/30	11/12/30	Alopecia. Complete recovery had taken place on 5/1/31.
29205	10/11/30	33 days	12·6 kg.	29368	20/11/30	—	Goat No. 29205, was found dead on 11/12/30. Cause of death unknown. Kid developed on symptoms of alopecia.

Goat 29205.—This animal kidded on 20.11.30 (kid 29368). From 10.11.30 up to the time of death from an unknown cause on 11.12.30 it had received 12·6 kilograms of the dry "bitterkarroo".

Goat 29205 was in perfect health up to 11.12.30 and was found dead in the stable the next morning.

Post-mortem Appearance.—Heart in diastole with both ventricles markedly distended with coagulated blood: marked hyperaemia and slight oedema of the lungs; degenerative changes in the liver; at the junction of the intermediate zone and medulla of the kidney there was deposition of a gritty material; haemorrhages in the peritracheal tissues; a slight chronic duodenitis and jejunitis.

Histology.—Liver: Slight hyperaemia; severe fatty changes more marked at the peripheri. Kidneys: Marked fatty changes; peculiar bodies in medulla of unknown significance.

Kid 29368.—This animal remained perfectly healthy in spite of the fact that it was suckled by its mother, which was drenched in the same way as goats 29203 and 29214 for three weeks.

Discussion.

Four pregnant goats were employed in this experiment (Table II), with the result that two typical cases of alopecia in kids were produced. One animal aborted on the twelfth day of the experiment and one died from an unknown cause three weeks after it had given birth to a normal kid. It will be noticed from Table II that 12·4 kg. and 23·2 kg. of the dry plant sufficed to produce the disease, while in another case was 12·6 kg. apparently below the minimum toxic dose necessary for the production of the disease.

EXPERIMENT III.

To determine whether the Colesberg "bitterkarroo" (*Chrysocoma tenuifolia* Berg.) will produce alopecia in kids.

TABLE III (Experiment III).
Dried Colesberg "Bitterkarroo".

Goat No.	Date of which dosage commenced.	Period of dosage.	Quantity of dry plant received.	Kid No.	Date of birth.	Date of appearance of alopecia symptoms.	Remarks.
29208	3/11/30	2 days	800 g.m.	—	—	—	Developed a profuse diarrhœa on 4/11/30 and aborted on 10/11/30. Recovered after treatment.
29210	3/11/30	2 days	800 g.m.	—	—	—	Profuse diarrhœa on 4/11/30, and died during the night of 5/11/30.
29219	3/11/30	2 days	800 g.m.	—	—	—	Profuse diarrhœa on 4/11/30, and died during the night of 4/11/30.
29206	6/11/30	19 days	1·4 kg.	{29357 29358}	16/11/30	—	A profuse diarrhœa developed after the animal had received 200 gm. of the plant on two consecutive days. After treatment with limewater and raw linseed oil recovery took place. Subsequently the animal received 1,000 gm. as described in the experiment. One of the kids died on 17/11/30, and the other one had to be discharged as the mother had no milk.
29211	6/11/30	60 days	6·7 kg.	29374	6/12/30	—	The mother developed a diarrhœa which disappeared after treatment The kid developed no symptoms of alopecia up to 5/1/31.
29212	6/11/30	35 days	3·95 kg.	29370	30/11/30	—	Both mother and kid developed a diarrhœa. No symptoms of alopecia appeared.
29218	6/11/30	2 days	400 gm.	—	—	—	On 8/11/30, the animal developed a pronounced diarrhœa and died during the night of 9/11/30.

The plant was collected in the flowering stage during October on the farm Springfontein, Colesberg District. Another attempt was made to persuade the experimental animals to take the plant voluntarily, but this had to be abandoned, as after three days' starvation they still refused to take the fresh plant.

Goat 29208.—On each of two consecutive days this animal received 400 grams of the dry plant.

Result.—Six hours after the second dose the animal exhibited a profuse diarrhœa associated with pronounced straining, complete inappetence, and drowsiness. An almost hairless kid was aborted, with the result that the animal had to be discharged from this experiment. Respiration and pulse accelerated. On each of three consecutive days she received a mixture of 200 c.c. of limewater and 100 c.c. of raw linseed oil, and had completely recovered in a week. The total quantity of dry plant received was 800 grams.

Goat 29210.—Received 400 grams of the dry plant on each of two consecutive days.

Result.—Six hours after the second dose a profuse diarrhœa set in. Tympanites, apathy, salivation, straining, and an accelerated pulse and respiration were the most outstanding symptoms. A mixture of 200 c.c. of limewater and 100 c.c. raw linseed oil was of no avail, death occurring sixty hours after the commencement of the experiment. The total quantity of dry plant received was 800 grams.

Post-mortem Appearances.—General cyanosis; numerous subepicardial haemorrhages; heart in diastole and the ventricles and auricles distended with coagulated blood; hyperaemia and oedema of the lungs; hyperaemia of the liver; atrophy of the spleen; an acute catarrhal gastro-enteritis. The uterus contained an almost mature foetus.

Goat 29219.—This animal, after having received 400 grams of the dry plant on each of two consecutive days, developed the above-described symptoms and died 40 hours after the commencement of the experiment. The total quantity of dry plant received was 800 grams.

Post-mortem Appearances.—General cyanosis; heart in diastole and both ventricles distended with coagulated blood; numerous subendocardial haemorrhages in left ventricles; hyperaemia and slight oedema of the lungs; hyperaemia of the liver; haemorrhages in the abomasum; an acute catarrhal enteritis.

The uterus contained an almost mature foetus.

As the Colesberg "bitterkarroo" proved to be more toxic than the Willowmore specimen, the following pregnant goats were drenched with smaller quantities of the plant.

Goat 29206—

6.11.30.—200 grams of the dry plant.

7.11.30.—200 grams of the dry plant.

8.11.30.—Pronounced diarrhœa and other symptoms as previously described. Treated with raw linseed oil and limewater.

9.11.30.—Marked improvement. Again treated.

10.11.30.—Completely recovered.

11.11.30-16.11.30.—50 grams of dry plant daily.

16.11.30.—Twin kids born (Nos. 29357 and 29359).

17.11.30.—100 grams of the dry plant.

18.11.30.—100 grams of the dry plant.

19.11.30.—Slight diarrhœa; inappetence and apathy; 100 grams of the dry plant.

20.11.30.—Profuse diarrhœa; treated with raw linseed oil and limewater as previously described.

21.11.30.—Recovering.

22.11.30.—Completely recovered; 100 grams of the dry plant.

23.11.30-25.11.30.—100 grams of the dry plant daily.

The animal was in a poor condition with a consequent low milk yield, with the result that she had to be discharged from the experiment.

The total quantity of dry plant received was 1,400 grams in the course of nineteen days.

Kid 29357.—This animal, together with kid 29368, had to be partly hand-reared as their mother (goat 29206) had very little milk. As was expected on account of the little mother's milk obtained, this kid developed no symptoms of alopecia.

Kid 29358.—It died twenty-four hours after birth, the post-mortem revealing an acute catarrhal duodenitis and jejunitis.

Goat 29211—

6.11.30.—200 grams of the dry plant.

7.11.30.—200 grams of the dry plant.

8.11.30.—Diarrhœa with the accompanying symptoms.

9.11.30–10.11.30.—Treated with raw linseed oil and limewater as previously described.

11.11.30.—Completely recovered.

11.11.30–5.12.30.—50 grams of the dry plant daily.

6.12.30.—Gave birth to a normal kid (No. 29374); 300 grams of the dry plant.

8.11.30.—300 grams of the dry plant.

9.11.30.—400 grams of the dry plant.

10.12.30.—400 grams of the dry plant.

11.12.30.—Pronounced diarrhœa with the accompanying symptoms; received a mixture consisting of 40 c.c. raw linseed oil, 100 c.c. limewater, and 1 gram tannic acid.

12.12.30.—Pronounced improvement.

13.12.30.—Pronounced improvement.

14.12.30.—Completely recovered.

15.12.30.—300 grams of the dry plant.

17.12.30.—Slight diarrhœa.

18.12.30.—Slight diarrhœa; 200 grams of the dry plant.

19.12.30.—Slight diarrhœa.

20.12.30.—300 grams of the dry plant.

22.12.30.—300 grams of the dry plant.

23.12.30.—300 grams of the dry plant.

24.12.30.—300 grams of the dry plant.

27.12.33.—300 grams of the dry plant.

29.12.30–5.1.31.—Daily 300 grams of the dry plant.

The animal received a total of 6·7 kilograms of the dry plant in the course of two months.

Kid 29374.—It developed no symptoms of alopecia.

Goat 29212—

6.11.30.—200 grams of the dry plant.

7.11.30.—200 grams of the dry plant.

8.11.30.—Diarrhœa with its accompanying symptoms; treated with a mixture of raw linseed oil, limewater and tannic acid as described before.

9.11.30.—Improved; again treated.

10.11.30.—Improved; again treated.

11.11.30.—Complete recovery.

11.11.30–30.11.30.—50 grams of the dry plant daily.

30.11.30.—Gave birth to a normal kid (No. 29370).

1.12.30.—150 grams of the dry plant.

2.12.30.—150 grams of the dry plant.

3.12.30–10.12.30.—300 grams of the dry plant daily.

11.12.30.—Pronounced diarrhœa with its accompanying symptoms; treated with a mixture of raw linseed oil, limewater, and tannic acid.

12.12.30.—Marked improvement.

13.12.30.—Improving.

14.12.30.—Complete recovery, but poor in condition with a consequent low milk yield.

As this animal was in such a poor condition, it was decided to discontinue the drenching. It received 3·95 kilograms of the dry plant in the course of thirty-five days.

Kid 29370 (born on 30.11.30).—On 13.12.30 the kid developed a pronounced diarrhœa and showed apathy and inappetence. From 15.12.30 rapid improvement set in until complete recovery on 17.12.30.

No symptom of alopecia appeared.

Goat 29218—

6.11.30.—200 grams of the dry plant.

7.11.30.—200 grams of the dry plant.

8.11.30.—Pronounced diarrhœa and listlessness, pulse extremely accelerated and weak; respiration hurried.

9.11.30.—Died previous night.

Post-mortem Appearances.—Advanced decomposition; an acute catarrhal gastro-enteritis; an almost full-grown foetus in uterus.

Result.

Seven pregnant goats were engaged in this experiment (Table III). The Colesberg "bitterkarroo" proved to be much more toxic than the Willowmore variety, with the result that one animal aborted and three died. The milk yield of two of the goats was very low. Unfortunately the conclusions concerning alopecia in this experiment have to be drawn from one case (No. 29211) only, and this was negative.

In the case of kid 29370 (mother goat 29212) a pronounced diarrhœa developed at the age of twelve days, and had the drenching of its mother not been discontinued from the fourth day after birth, the possibility exists that it would have developed alopecia, as diarrhœa sometimes precedes the shedding of the hair.

Experiment IV.

To ascertain whether it is possible to produce alopecia in kids by drenching their mothers from the day of parturition.

Goat 29215.—Kidded on 13.11.30 (kid 29355). From 13.11.30 to 8.12.30 this animal received 11·6 kilograms of the dry Willowmore "bitterkarroo".

Kid 29355.—It developed no symptoms of alopecia.

Goat 29209.—Kidded on 15.11.30 (Kid 29356).

15.11.30 to 9.12.30 this goat received 11·2 kilograms of the Willowmore "bitterkarroo".

Kid 29356.—It developed no symptoms of alopecia.

Result.

From the above it would appear that for the production of alopecia in kids it is necessary that they be exposed to the effects of the toxin during a part of their intra-uterine life. This point will be discussed at the conclusion of this article.

Experiment V.

To determine whether alopecia can be produced in kids by drenching them with the Willowmore "bitterkarroo".

Kid 29246 (Mother 29216).—This animal, which was born on 1.11.30, received the following amounts of the dry plant.

5.11.30–8.11.30.—10 grams daily.	22.11.30.—40 grams.
10.11.30.—15 grams daily.	24.11.30.—45 grams.
14.11.30.—20 grams.	25.11.30.—50 grams.
15.11.30.—20 grams.	26.11.30.—50 grams.
17.11.30.—25 grams.	27.11.30.—60 grams.
18.11.30.—25 grams.	28.11.30.—80 grams.
19.11.30.—30 grams.	29.11.30.—90 grams.
20.11.30.—30 grams.	1.12.30.—100 grams.
21.11.30.—35 grams.	2.12.30.—100 grams.

3.12.30–8.12.30.—120 grams daily.

It, therefore, received 1·62 kilograms of the dry plant in the course of thirty-four days.

Result.

Complete negative.

Kid 29361 (mother 29217).—It was born on 15.11.30 and was drenched with the following amounts of the dry Willowmore " bitterkarroo ".

19.11.30.—10 grams.
20.11.30.—10 grams.
21.11.30.—15 grams.
22.11.30.—20 grams.
24.11.30.—25 grams.
25.11.30.—30 grams.
26.11.30.—30 grams. Slight diarrhœa.
27.11.30.—30 grams. Slight diarrhœa.
28.11.30.—40 grams. Diarrhœa improving.
29.11.30.—50 grams. Diarrhœa worse.
1.12.30.—60 grams. Diarrhœa improving.
2.12.30.—60 grams. Diarrhœa improving.
3.12.30.—80 grams. Complete recovery.
4.12.30.—80 grams. Vomited after being drenched.
5.12.30.—100 grams. Vomited after being drenched.
6.12.30.—80 grams. Vomited after being drenched.
8.12.30.—80 grams. Vomited after being drenched.
9.12.30.—80 grams. Vomited after being drenched.
10.12.30.—80 grams. Vomited after being drenched.
11.12.30.—100 grams. Vomited after being drenched.
12.12.30.—100 grams. Vomited after being drenched.
13.12.30.—100 grams. Vomited after being drenched.
15.12.30.—100 grams. Vomited after being drenched.

This kid received 1·36 kilograms of the dry plant during a period of twenty-six days without developing any symptoms of alopecia, the only noticeable ill-effect being a transitory diarrhœa. Emesis was probably due to the large amount of fluid given.

Result.

None of the kids developed symptoms of alopecia, in spite of the fact that they received maximum amounts of the dry plant over a long period.

Experiment VI.

To ascertain whether Merino lambs are susceptible to alopecia.

For this purpose four pregnant Merino ewes were drenched with the dry Willowmore " bitterkarroo ". The dry plant as such was offered to the sheep, but was refused. Small quantities were then mixed with dry lucerne-hay, but still the animals bluntly refused to take any of the mixture. Hence it was resorted to drenching.

As nothing was known about the effects of the plant on Merino sheep, it was decided first to determine these before commencing the actual experiment. To this end sheep 38412 was drenched with the dry plant.

Sheep 38412—

13.10.30—400 grams.
14.10.30.—400 grams.
15.10.30.—400 grams.
16.10.30.—400 grams in the morning and 400 grams in the afternoon.
17.10.30.—400 grams in the morning and 400 grams in the afternoon.
18.10.30.—Slight diarrhœa. 400 grams.

20.10.30.—400 grams in the morning and 400 grams in the afternoon. Slight diarrhœa.

21.10.34.—400 grams; diarrhœa; listlessness; accelerated pulse and respiration; thirst.

TABLE IV (Experiment VI).

The Effect of Willowmore "Bitterkarroo" on Merino Ewes and their Lambs.

Ewe No.	Date on which dosage commenced.	Period of dosage.	Quantity of dry plant received.	Lambs No.	Date of birth.	Date of appearance of alopecia symptoms.	Remarks.
29199	27/10/30	6 days	2·8 kg.	29243	29/10/30	—	On 1/11/30, the ewe developed a diarrhœa and died on 3/11/30. Lamb developed no symptoms of alopecia.
29196	29/10/30	7 days	2·8 kg.	29242	3/11/30	—	On 5/11/30, a slight diarrhœa set in, which disappeared after treatment. On 8/11/30, another 200 grams of the plant were given with the result that diarrhœa again developed. On account of the lamb had to be discharged from the experiment on 17/11/30. On the morning of 19/11/30 the ewe was found dead.
29197	29/10/30	7 days	2·8 kg.	29241	3/11/30	—	On 5/11/30 diarrhœa developed and, in spite of treatment, death accurred on 8/11/30. The lamb died from a bilateral acute lobar pneumonia during the night of 13/11/30, without having developed any symptoms of alopecia.
29195	29/10/30	7 days	2·8 kg.	Not numbered	4/11/30	—	Ewe had no milk and was discharged from the experiment. It developed an acute diarrhœa on 6/11/30, and died during the night of 7/11/30. The lamb was found dead on the morning of 6/11/30.

22.10.30.—400 grams in the morning and 400 grams in the afternoon; diarrhœa pronounced.

23.10.30.—Pronounced diarrhœa and straining; general weakness; pulse extremely accelerated; respiration hurried; losing condition; drenching discontinued.

These symptoms culminated in death during the night of 25.10.30.

Post-mortem Appearances.—Decomposition too advanced to discern any definite lesions.

Result.

The animal developed a slight diarrhœa after having received 2·8 kilograms of the dry plant in the course of five days. The doses for the pregnant animals were based on the effects of the above quantities of the plant on this sheep. In order to be able to draw definite conclusions from the results of this experiment the ewes will have to receive maximum quantities of the plant. As the above sheep apparently tolerated 2·8 kilograms of the dry plant given in five days with very slight ill-effects, it was decided to give the pregnant ewes 400 grams daily.

Ewe 29199—

27.10.30.—400 grams.

28.10.30.—400 grams.

29.10.30.—400 grams. Gave birth to a normal lamb (No. 29243).

30.10.30.—400 grams.

31.10.30.—400 grams in the morning and 400 grams in the afternoon.

1.11.30.—400 grams; slight diarrhœa in the afternoon.

2.11.30.—Diarrhœa; salivation; pronounced thirst; straining; accelerated pulse and respiration.

3.11.30.—Died.

Post-mortem Appearances.—General cyanosis; numerous sub-epicardial haemorrhages; degeneration of myocard; hyperaemia and slight oedema of the lungs; pronounced degeneration of the liver and kidneys; atrophy of the spleen; pronounced acute catarrhal enteritis affecting the whole of the small and big intestine.

Lamb 29243 (born 29.10.30).—It was suckled by its mother for five days only, and as a result of her death it had to be fed on cow's milk.

Result.

No symptoms of alopecia developed.

Ewe 29196—

29.10.30–4.11.30.—400 grams daily.

3.11.30.—Gave birth to a normal lamb (No. 29242).

5.11.30.—Slight diarrhœa; treated with a mixture of limewater and raw linseed oil.

6.11.30.—Diarrhœa; treated as above.

7.11.30.—Recovered.

8.11.30.—200 grams.

10.11.30.—Diarrhœa; limewater and raw linseed oil.

11.11.30.—Diarrhœa improving, but condition poor.

12.11.30.—Milk yield very low; condition poor.

13.11.30–14.11.30.—Milk yield very low; condition poor.

This animal, with its lamb, No. 29242, were discharged on 17.11.30, as it had very little milk. On the morning of 19.11.30 it was found dead.

Post-mortem Appearances.—Anaemia; cachexia; hyperaemia; oedema and emphysema of the lungs, with a large amount of coagulated blood in the bronchi and trachea; heart in systole.

Lamb 29241 (born on 3.11.30).—As its mother (Ewe 29196) had very little milk, it had to be partly hand-reared.

Result.

This animal developed no symptoms of alopecia.

Ewe 29197—

29.10.30–4.11.30.—400 grams daily.

3.11.30.—Gave birth to a normal lamb (No. 29241).

5.11.30.—Slight diarrhœa; treated with a mixture of limewater and raw linseed oil.

6.11.30.—Diarrhœa; treated as above.

7.11.30.—Diarrhœa; treated as above.

8.11.30.—Pronounced diarrhœa; vomiting; apathetic; accelerated and weak pulse; hurried respiration.

Died at 9 a.m.

Post-mortem Appearances.—General cyanosis; marked hydrothorax; both ventricles of the heart distended with coagulated blood; hyperaemia and oedema of the lungs with ruminal contents in the trachea and bronchi; slight haemonchosis; an acute catarrhal enteritits affecting both the small and big intestine.

Histology.—Heart, liver, spleen, and kidneys: Negative.

Lamb 29241.—Died from a bilateral acute lobar pneumonia during the night on 13.11.30 without having developed any symptoms of alopecia.

Ewe 29195—

29.10.30–4.11.30.—400 grams daily.

4.11.30.—Gave birth to a normal lamb. Animal was discharged from the experiment, as it had no milk.

On 6.11.30 it developed a diarrhoea, and in spite of treatment with limewater and raw linseed oil it died during the night of 7.11.30.

Post-mortem Appearances.—General cyanosis; hyperaemia and oedema of the lungs; slight hydroperitoneum and hydrothorax; both auricles and ventricles of the heart distended with coagulated blood; atrophy of the spleen; acute catarrhal abomasitis duodenitis, typhlitis and colitis; a croupous jenunitis.

Histology.—Liver, heart, spleen, and kidney: Negative.

Lamb (died before it was numbered).—It was found dead on the morning of 6.11.30, and the post-mortem revealed an acute catarrhal gastritis.

Result.

The results of this experiment as far as alopecia is concerned are inconclusive, owing to the fact that the quantities of the plant given had pronounced toxic effects on the sheep, with the result that they either had very little or no milk for the lambs or died. The dry plant proved to be more toxic to sheep than to goats.

The Effects of Potassium Iodide on Pregnant Angora Goats.

It is of interest to submit on the next page a table of the abortions which occurred in the experimental groups of Angora goats receiving potassium iodide. The calculations of the daily doses of potassium iodide for the pregnant goats was based upon the work of Kelly* (1925).

* Kelly, F. C. (1925): The Influence of Small Quantities of KI on the Assimilation of N.P. and Ca in the Growing Pig. *Biochem. Jnl.*, 19, 559-568.

TABLE V.

Effects of Potassium Iodide on Pregnant Angora Goats.

Goat No.	Amount of Potassium Iodide per day.	Period of dosage.	Total amount of Potassium Iodide received.	Additional treatment.	Date dosage with Potassium Iodide was commenced.	Date of abortion.
	Grams.	Days.	Grams.			
1	0·1	8	0·8	Ingested daily approximately 4 lb. of "bitterkarroo" per head (figures from the Willowmore Experiment I)	12/8/30	19/8/30
2	0·1	9	0·9		12/8/30	20/8/30
3	0·1	12	1 2		12/8/30	23/8/30
4	0·05	16	0·8		1/9/30	16/9/30
5	0·05	16	0 8		1/9/30	16/9/30
6	0 1	10	1·0	Ingested daily approximately 2 lb. of flowers and leaves of Bothablombos (figures from the Willowmore Experiment II)	12/8/30	21/8/30
7	0·1	12	1·2		12/8/30	23/8/30
8	0·05	16	0 8		1/9/30	16/9/30
9	0·1	17	1 7	These animals belong to the group of 20 pregnant goats which were running with the remainder of the flock (figures from the Willowmore Experiment III)	12/8/30	28/8/30
10	0·1	20	2·0		12/8/30	1/9/30
11	0·1 gm. from 12/8/30–31/8/30 0·05 gm. from 1/9/30–11/9/30	31	2·55		12/8/30	11/9/30
12	0·1 gm. from 12/8/30–31/8/30 0·05 gm. from 1/9/30–14/9/30	34	2 7		12/8/30	14/9/30
13	0·1 gm. from 12/8/30–31/8/30 0·05 gm. from 1/9/30–14/9/30	34	2 7		12/8/30	14/9/30

The facts contained in the above table must be viewed in the light of the following information. The total number of pregnant goats employed in Experiment I was twenty-four and of these eight received potassium iodide. Of these twenty-four animals six aborted, five of which were engaged in the potassium iodide group and one in the salt-bonemeal group. Hence the abortions in the the Bothablombos group. The abortions in the potassium iodide group therefore amounted to 43 per cent., whereas in the rest of the animals in Experiment II they amounted to 10·5 per cent.

In Experiment II nineteen pregnant goats were engaged and of these seven belonged to the potassium iodide group. Of the seven potassium iodide animals three aborted, whereas in the rest of the animals one abortion occurred in the salt-bonemeal group and one in the Bothalombos group. The abortion in the potassum group therefore amounted to 43 per cent., whereas in the rest of the animals in Experiment II they amounted to 10·5 per cent.

In Experiment III twenty pregnant goats, which received potassium iodide as described before, were allowed to run with the flock of pregnant goats. Of these animals five aborted, the percentage of abortions being 25 per cent.

The twenty pregnant goats receiving 5 grams of sulphur daily, and running with the twenty potassium iodide goats in the same flock, as well as the flock of three hundred pregnant goats, served as controls to the animals receiving potassium iodide. No abortions occurred in the sulphur group, whereas only six occurred in the flock of three hundred pregnant goats.

From the above it is evident that the percentage abortions in the potassium iodide-bitterkarroo group in comparison with the other potassium iodide groups is abnormally high. As the bitterkarroo itself is inclined to produce abortions this high percentage is quite conceivable.

With regard to the abortions in the flock cotyledonosis must be taken into consideration.

Discussion.

It has been definitely proved both during the course of field experiments in the Willowmore district and at Onderstepoort that *Chrysocoma tenuifolio* is the cause of alopecia in kids and the lambs of cross-breeds of sheep. This plant is ingested by the pregnant and lactating animals and the " toxin " producing alopecia eliminated in the milk, which is ingested by the kids and lambs. It is in this way that the latter animals contract alopecia. Furthermore, it has been shown that the sun-dried plant is also capable of causing the disease. In the experimental animals the earliest case of alopecia occurred in a three-day-old kid and the latest case in an eleven-day-old kid.

Prevention.

In order to prevent the disease it is obvious that the pregnant and suckling animals should be kept away from veld where there is luxurious growth of the " bitterkarroo ". On farms where the mountain grazing is of a good quality, and where there is a sufficient water

supply for the growing of green foodstuffs, this method of preventing the disease can be applied with great success. The position is different on farms where the above conditions do not exist. It is on the latter farms that the owners completely discontinued kidding and lambing, as they suffered losses up to 100 per cent.

It was previously mentioned that many farmers combat the disease with great success by removing the pregnant animals from " bitterkarroo-veld " fourteen days before kidding and lambing and not returning them to such veld before the kids and lambs are fourteen days old. There is no experimental evidence to bear this out, but both from field observations and experimental results it appears that after the age of fourteen days kids and lambs will not, or very rarely, develop the disease. Very few cases are known where three and four weeks' old kids and lambs suffered from alopecia.

Another method practised by farmers with a fair degree of success in preventing alopecia is the partly milking of the suckling goats and ewes. This milk is taken by the natives without any deleterious effects.

THE SUSCEPTIBILITY OF THE YOUNG OF DIFFERENT CLASSES OF STOCK TO ALOPECIA.

Up to the present alopecia has been known to occur only in kids and lambs of Blackhead Persians and all cross-breeds of sheep. No cases have been reported in Merino lambs or any species of domestic animals, except those mentioned in Merino lambs by Van Rensburg (1925). Nothing definite can be said concerning the susceptibility of Merino lambs to alopecia, as no Merino sheep are kept in the alopecia areas. The results of the experiment conducted at Onderstepoort to determine the susceptibility of Merino lambs to alopecia are inconclusive.

THE SUSCEPTIBILITY OF FULL-GROWN SHEEP AND GOATS TO THE EFFECTS OF THE " BITTERKARROO ".

Merino ewes proved to be more susceptible than Angora goats. A noteworthy fact is that in the course of the Willowmore experiments it was found that when the fresh flowering " bitterkarroo " alone was fed to the Angora goats at the rate of four pounds per head per day the animals developed a fatal diarrhœa, whereas the same amount of plant, when supplemented by lucerne-hay and green barley, had no ill-effects, with the exception of loss in condition. It might be stated that at Onderstepoort excellent results were obtained by treating the diarrhœa with a mixture of 100 c.c. raw linseed oil, 100 c.c. limewater and 1·0 gram tannic acid. Both at Willowmore and at Onderstepoort a large number of animals, which suffered from diarrhœa caused by the " bitterkarroo ", were treated with limewater and raw linseed oil, and with a mixture of limewater, raw linseed oil and tannic acide and the latter mixture was found to be far superior to the former.

THE DIRECT EFFECTS OF THE "BITTERKARROO" ON KIDS.

Two four-day-old kids were drenched daily with comparatively large amounts of the dry plant without any apparent ill-effects, one of the kids showing a slight transient diarrhœa. No symptoms of alopecia developed. From these results it appears that either (*a*) the "alopecia-toxin" is not present as such in the plant, but has to be formed or modified by the mother goat or ewe, or (*b*) for the appearance of alopecia it is essential that the "alopecia-toxin" must act on the fœtus for a certain period, or (*c*) the amount of plant given, although maximum quantities were administered, was too small. In the last case it must be remembered that the mother-goats consumed approximately four pounds of the fresh plant per head per day and the possibility exists that the "alopecia-toxin" might be excreted in the milk in a concentrated solution. It is obvious that such enormous amounts of plant cannot be administered to kids and lambs.

In this connection it might be stated that two goats were drenched for twenty-five days with the dry plant from the day they kidded and their young did not develope alopecia.

THE RELATIVE TOXICITY OF THE COLESBERG AND WILLOWMORE "BITTERKARROO" TO FULL-GROWN ANGORA GOATS.

Both varieties of the plant were utilized in the dry state and in the flowering stage. The Colesberg variety proved to be much more toxic than that obtained from Willowmore. The only noticeable difference in the two varieties was that the Colesberg plant showed a much more luxuriant growth than the Willowmore variety. The rainfall three months prior to the collection of the plant was much higher in the Willowmore than in the Colesberg District. The soil on which the "bitterkarroo" was collected in the Colesberg District is of a much superior quality than that of the farm on which the Willowmore plant was collected.

THE RELATION BETWEEN ALOPECIA AND THE DIARRHŒA.

In a number of cases of alopecia in kids and lambs the alopecia was preceded by a diarrhœa, but in the great majority of cases, alopecia was the first noticeable symptom. Apparently the cause of diarrhœa in the kids and lambs is a two-fold one, namely, a toxin contained in the "bitterkarroo" plant and excreted in the milk and one caused by the mechanical irritation of the ingested hair. After the appearance of alopecia these two factors will act as synergists. Most probably the above toxin is also responsible for the diarrhœa in the full-grown goats and sheep. Whether this "diarrhœa-producing toxin" is identical with the "alopecia-producing toxin" is an open question. The "diarrhœa-producing toxin" complicates the alopecia experiments, as large amounts of the plant have to be given in order to produce the disease, and on the other hand a sub-toxic dose must be given, as it is essential for the suckling sheep and goats to have a high milk-yield.

POTASSIUM IODIDE AND ITS RELATION TO ABORTION.

A summary of the abortions in all the different experiments at Willowmore and in the controls is given in Table VI.

CONCLUSIONS.

(1) *Chrysocoma tenuifolia* Berg. ("bitterkarroo"), has been established as the cause of alopecia in kids and lambs. The "toxin" is eliminated through the milk.

(2) The disease can be successfully prevented by avoiding *Chrysocoma tenuifolia* veld for a period of fourteen days prior to, and after kidding and lambing.

(3) Up to the present alopecia has been known to occur in kids of all breeds of goats, and all cross-breeds of sheep. Cases in Merino sheep have been reported.

(4) *Chrysocoma tenuifolia* when ingested in large quantities produces abortions in pregnant sheep and goats as well as symptoms of severe gastro-intestinal irritation.

(5) Two four-day old Angora kids drenched with large amounts of *Chrysocoma tenuifolia* for a period of twenty days developed no symptoms of alopecia.

TABLE VI.

The Effect of Potassium Iodide on Pregnant Angora Goats.

Exp. No.	Number of goats in experiment.	Number of goats which received KI.	Total number of goats which received KI.	Number of KI goats which aborted.	Total Number of KI goats which aborted.	Total Number of goats in the experiment + controls.	Percentage abortions in KI groups.	Percentage abortions in rest of experimental goats and controls.
I	24	8	35	5	13	383	37	2·3
II	19	7		3				
III	20	20		5				
IV	20	None (all received sulphur)		None				

(6) The Colesberg *Chrysocoma tenuifolia* was found much more toxic to sheep and goats than the Willowmore variety.

(7) A point to be investigated is the relation between the "diarrhœa-toxin" and "alopecia-toxin" contained in *Chrysocoma tenuifolia*.

(8) The abortions in the goats receiving potassium iodide amounted to 37 per cent., whereas in the rest of the experimental animals and the controls they amounted to 2·3 per cent. only.

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LITERATURE.

Reko (1928) described a disease in horses, mules, cattle, sheep and goats occurring in the southern parts of the United States of America, in the course of which the animals shed their coat. In advanced cases of this disease there is pronounced inflammation of the joints, which ultimately causes death. Stock-owners incriminate the "loco-weed" *(Astragalus lambertii, Astragalus molissimus* and *Cystium diphysium)*. Reko has not seen the disease where *Astragalus* sp. do not occur.

A similar disease occurs in Mexico in animals which ingest *Tamarindus indica* over long periods. As soon as the animals are prevented from feeding on the plant the hair commences growing. The aborigines of South Mexico daily partake of the ground Tamarindus seeds to depilate themselves.

These seeds are used to breed the small hairless Chihuahua dogs. Pneumonia and dropsy frequently are the cause of death in these hairless animals.

Furthermore, thallium salts are active poisons and may lead to alopecia (Ward, 1930). Since 1930 thallium has been used in America as a proprietary rat-poison and thallium poisoned grain for the control of prairie dogs, with the result that many losses have occurred in sheep due to the ingestion of this poisoned grain. It was found that sub-lethal doses of thallium down to 9 milligrams per Kg. body-weight cause alopecia in sheep.

Landauer (1931) found that treatment of mature cockerels and pullets with thallium acetate is followed by a diffuse loss of feathers. Thallium poisoning is furthermore referred to by Buschke and Peiser (1932), Ginsberg and Nixon (1932), Landauer (1931), Leschke (1931), Ward (1930), and others.

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Section V.

Sex Physiology.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 3, Number 2, October, 1934.

Studies in Sex Physiology No. 12: The Situation of the Developing Foetus and the Relationship between the Pregnant Horn and the corresponding Corpus Luteum Verum.

By

H. H. CURSON, Dr.Med.Vet., F.R.C.V.S., Veterinary Research Officer, Onderstepoort, and

G. S. MARÉ, B.Sc.(Agric.), Sheep and Wool Research Officer, Grootfontein School of Agriculture, Middelburg, C.P.

Introduction.

In Study 10 (Curson and Quinlan) the situation of the foetus was investigated in a series of 41 pregnancies (33 days 4 hours to 108 days 16 hours) supervised by Mr. G. S. Maré, of Grootfontein School of Agriculture, C.P.

It is now possible to extend this series as follows: Pregnancies of (*a*) 20 days; (*b*) 25 days; (*c*) 30 days; (*d*) 115 days; (*e*) 125 days; (*f*) 135 days; and (*g*) 145 days. As will be evident from a perusal of the table accompanying the study in question, specimens (*a*), (*b*), and (*c*) are earlier than shown formerly, while (*d*), (*e*), (*f*), and (*g*) are later than previously given.

Details regarding the new specimens may be tabulated thus:—

Serial number of foetus.	Official number of ewe.	Age of foetus.	Approximate total weight of unopened foetus.	Approximate weight of foetus. Gm.	Approximate size C.R. length. Cm.
F (*a*).....	A. 1 144	20 days	47* gm. (48·5)	specimen lost	—
G (*a*).....	D. 75	25 days	91* gm. (93·7)	,,	—
H........	A. 1 146	30 days	91* gm. (85·2)	0·62	1·6
		See data furnished in Study 10.			
42........	A. 1 152	115 days	2915* gm. (3260)	1490	26
43........	A. 1 145	125 days	5499* gm. (6220)	2810	33
44........	V. 12	135 days	5017* gm. (5260)	2780	35
45........	A. 1 143	145 days	4988* gm. (5140)	2790	35

* Figures supplied by Mr. Maré, who weighed the specimens *before* being placed in formalin. The figures in brackets are those determined at Onderstepoort *after* arrival of the material.

As will be seen in the figures, not only are the unopened uteruses shown in the dorso-ventral position (cervix being caudal), but the situation of the foetuses has been traced. Fig. 1 is a non-pregnant uterus which should be compared with Fig. 2 [uterus containing foetus F (*a*)].

Fig. 1.—Control: Non-pregnant uterus of sheep.
Fig. 2.—Uterus, 20 days pregnant, containing Foetus F (*a*).
Fig. 3.—Uterus, 25 days pregnant, containing Foetus G (*a*).
Fig. 4.—Uterus, 30 days pregnant, containing Foetus H.

Discussion of Figures.

As before, the situation of each foetus is clearly shown in the figures. The following facts emerge from an analysis:—

(*a*) All 7 pregnancies are *single*. This means that of a series of 48 pregnancies *in all*, 43 are single (see Study 10).

(*b*) Concerning *presentation*, 5 out of 7 cases are cranial, the direction in all foetuses being longitudinal. Of the entire series of 40 *undisturbed* single pregnancies (total sheep, 48), 25 are cranial and 15 are caudal.

(*c*) Regarding *position* the following relations are clear: 4 dorso-sacral (oldest foetuses) and 3 dorso-iliac (youngest foetuses), 2 of which were resting on their left side and 1 on its right side.

(*d*) With reference to *posture*, the general flexed state of the head and limbs was noted, as shown in the figures.

In this series of 7 pregnancies, the material had not been tightly packed in tins as before.

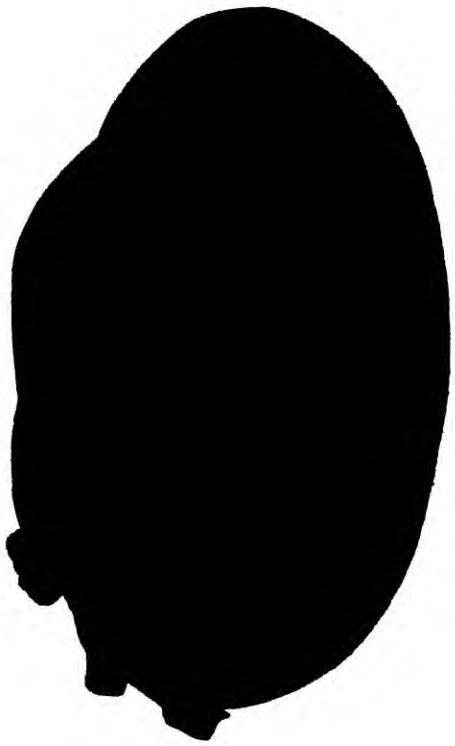

Fig. 5.—Uterus, 115 days pregnant, containing Foetus 42.

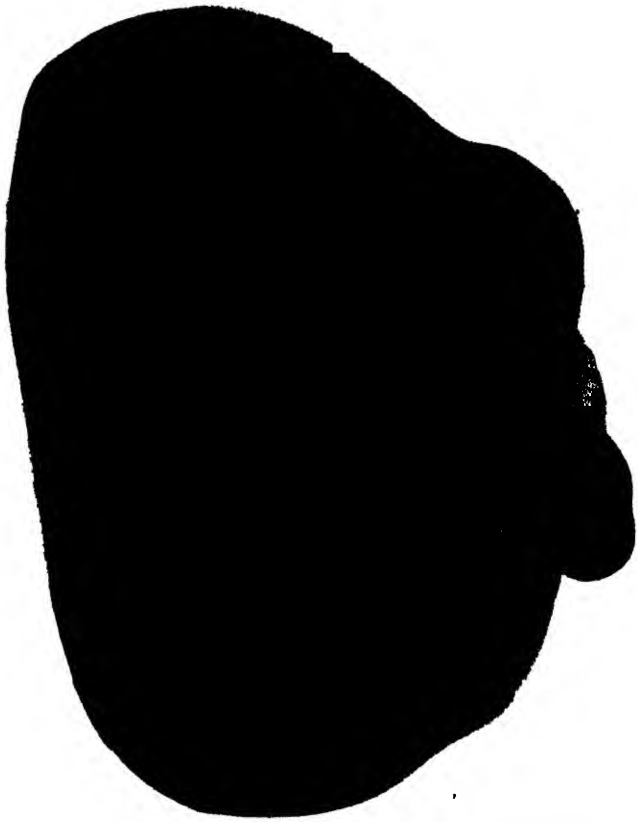

Fig. 6.—Uterus, 125 days pregnant, containing Foetus 43.

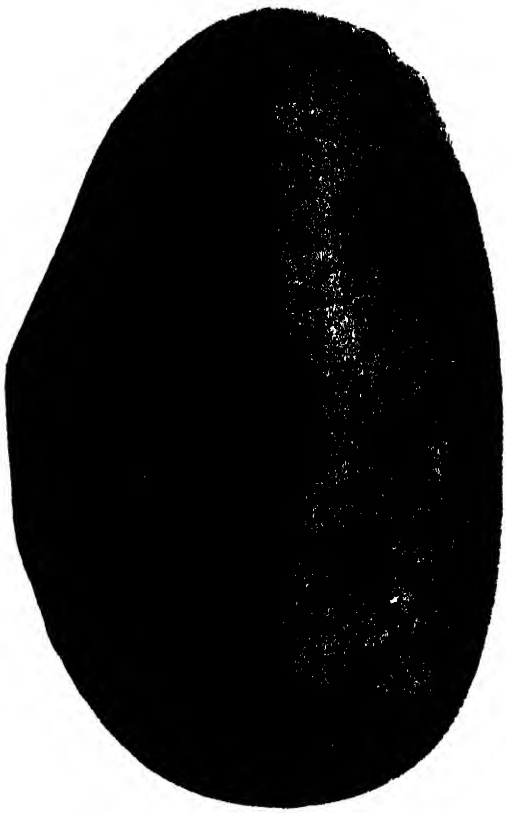

Fig. 7.—Uterus, 135 days pregnant, containing Foetus 44.

Fig. 3.—Uterus, 145 days pregnant, containing Foetus 45.

As to the relationship between the pregnant horn and the corresponding corpus luteum verum, details regarding the earlier genitalia (of Study 10) are recorded in Study 11 (Curson). It now remains to supply information on the additional genitalia recently furnished from Grootfontein.

Of the 7 sheep, the *usual* state of affairs pertained in all cases, namely, the foetus was situated in the horn corresponding with the ovary from which the ovum in question arose. In two cases [Nos. F (*a*) and 43] the *corpus luteum verum* and pregnant horn were on the left side and in the remaining cases [Nos. G (*a*), H, 42, 44, and 45] these structures were on the right side. There was thus no *migration* of the ovum. Of the entire series of 43 single pregnancies 41 foetuses developed on the same side as the ovulating ovary. This in 17 cases was on the left side and 24 on the right side.

Summary.

It is clear that this paper is merely an addendum to Study 10 (dealing with the situation of the developing Merino foetus *in utero*) and Study 11 (concerning the relationship between the pregnant horn and its associated *corpus luteum verum*).

Material Studied.

The females used in the foregoing observations were selected at random from the Merino flock maintained at the Grootfontein School of Agriculture. They were of mixed ages, ranging from 4-tooth to full-mouth. Different rams, bred in the Grootfontein Stud, impregnated the ewes.

Vasectomised teasers were placed among the flock once a day and all ewes showing oestrus were separated for service. Each ewe was held to facilitate proper service by the rams. In this way the exact hour of service of every ewe was known.

Both before service and during pregnancy the ewes grazed on Karroo veld, which was moderately good. All carcases were in a condition fit for human consumption.

At intervals the ewes were killed and the genital organs removed, the tract being severed at the posterior extremity of the cervix. The pregnant uteruses were generally weighed immediately on removal and afterwards placed in a 10 per cent. formalin solution and railed from Grootfontein to Onderstepoort.

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CURSON, H. H. (1934). Studies in Sex Physiology, No. 11: The relationship between a corpus luteum verum and the corresponding pregnant horn. *Id.* Vol 3, No. 1, p. 133.

Section VI.

Miscellaneous.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 3, *Number* 2, *October*, 1934.

Anatomical Studies No. 50.

A Comparison of Hamitic Longhorn, West African Shorthorn and Afrikander Cattle Particularly with regard to the Skull (1).

By H. H. CURSON, F.R.C.V.S., Dr.Med.Vet., Veterinary Research Officer, Onderstepoort, and Dr. H. EPSTEIN, Welverdiend.

INTRODUCTION.

It is intended first to summarise certain facts regarding each of the above three types of cattle (since they represent foundation types of African cattle); and then to indicate how the skulls vary, the skull, as is generally known, being the feature ot greatest importance in the study of ancestry. Thanks to Epstein (Dr. H.), much light has been thrown on the racial history of African Cattle ([2]), and it is his account of the Hamitic Longhorn ([3]) which is being followed in this paper.

([1]) This note could very well appear under *Studies in Native Animal Husbandry*, which series at present comprises the following:—

CURSON, H. H., THOMAS, A. D., AND NEITZ, W. O. (1930). 1. Notes on the Wankonde. *Jl. S.A.V.M.A.*, I (4).

CURSON, H. H., THOMAS, A D., AND NEITZ, W. O. (1931). 2. Proposed Plan of Investigation. *Jl. S.A.V.M.A.*, II (2).

CURSON, H. H., THOMAS, A. D., AND NEITZ, W. O. (1933). 3. Native Milking Pails. *Argeologiese Navorsing van die Nasionale Museum*, Bloemfontein.

DICKE, B. H. (1931). 4. Bantu and Cattle in the Northern Transvaal. *Jl. S.A.V.M.A.*, II (2).

THOMPSON, F. R. B. (1932). 5. Indigenous Cattle in the Transkeian Territories. *Jl. S.A.V.M.A.*, III (4).

GROENEWALD, J. W., AND CURSON, H. H. (1933). 6. A Note on Ovambo Cattle. *Onderstepoort Jl. Vet. Sc. and Anim. Indust.*, I (2).

BISSCHOP, J. H. R., AND CURSON, H. H. (1933). 7. Makalanga Cattle: A Representative Described. *Id.*

DART, R. A. (1933). 8. The Domesticated Animals of Pre-European South Africa. *Jl. S.A.V.M.A.*, IV (2).

EPSTEIN, H. (1934). 9. The West African Shorthorn. *Jl. S.A.V.M.A.*, V (3).

CURSON, H. H. (1934). 10. The West African Shorthorn (*Bos brachyceros*). *Id.*

([2]) His book, *The Origin of Africa's Indigenous Domestic Animals*, is unfortunately not yet published, but he kindly submitted Chapter IV on " The Cattle of Africa " to the Director of Veterinary Services for comment. Epstein believes that the West African Shorthorn and the Afrikander are representatives of the Brachyceros and Longhorned Zebu types respectively.

([3]) Neffgen (1904), in his description of the *Veterinary Papyrus of Kahun*, mentions that the oldest race of cattle was the " Langhornrasse " which was dominant during the " Alten Reiche ", i.e. *c.* 2830 B.C.–2530 B.C.

Hamitic or Egyptian Longhorned Cattle.

It is believed that the ancestor of this type was the giant horned indigenous wild ox of the Nile Valley, called by Hilzheimer *Bos primigenius Hahni, nova subspecies Hilzheimer*. Domestication took place probably before and during the Neolithic era of Egypt. At the end of the Neolithic era, there entered Egypt from Asia (via the Isthmus of Suez), cattle of an entirely different type, namely, the Brachyceros or Shorthorn.

In the meantime Hamitic migration carried the Hamitic Longhorn west along North Africa into Spain and Portugal, and descendants of these cattle are now to be seen in the Raza de Barroza, Raza Minhota, and Raza Alentejana* (Portugal) and cattle of Andulusia (Spain). From the Iberian Peninsula, Brazil imported cattle and the Franqueiro Breed there represents the Hamitic Longhorn. Great Britain too has representatives in the Black Cattle of Wales, West Highland Cattle, and Herefords. Other migrations in Africa were mainly in a southerly direction.

In Africa, the Hamitic Longhorn has disappeared from the Nile Valley, but cattle of the same type are to be found in the interior of Senegambia and Liberia (Mandingo cattle)† and in the inaccessible mountain valleys of Morocco. With regard to Egypt, Flower (1932) describes the present cattle population as follows:—

" *Domestic Ox.*

Bos taurus Linnaeus.

The domestic cattle of Egypt in my time were all of one shorthorned type from Alexandria to Aswan. Then followed in Lower Nubia an entirely cattleless country, except for a few animals imported into towns as El Derr and Wadi Halfa. In Upper Nubia from Dongola Province southwards cattle were again met with, but these could be referred to the humped species *Bos indicus*. I noted no domestic cattle in the Wadi Natron, and saw very few in Sinai.

When the importation of cattle from the Sudan to Egypt commenced in 1902 the large humped beasts with long up-standing horns were a matter of great interest to the fellaheen, all along the railway from Aswan north to Cairo. These oxen appealed to their minds as greater curiosities than giraffes or lions; after all, a wild animal, to people who were brought up to believe in the unicorn and the phoenix, might be of any shape or form, but they had definite ideas as to the necessary appearance of domestic beasts.

In Egypt, where the cattle literally live among the people, and often sleep in the same rooms, these animals are quiet and peaceable. I never found them aggressive, as on a few occasions I have found the humped cattle to be in Kordofan and on the Blue Nile."

* Da Costa (1931) considers this breed a relative of the Afrikander.

† Sir Harry Johnston (1906) describes these cattle and includes several good photographs.

West African Short-horned Cattle.

As mentioned above, at the end of the Neolithic era in Lower Egypt there arrived the Brachyceros cattle, and even during the period of the New Kingdom (*c.* 1530 B.C.–1050 B.C.) they were dominant ([4]). According to Epstein, " it is safe to assume that there lived in some part of Asia a local subspecies of *Bos primigenius*, in the domestic descendants of which those physical characteristics that, throughout the animal world, are found only in the state of domestication, and which in *Bos taurus* are called brachyceros, became fixed by mutation ".

As a result of the invasion by Brachyceros the Hamitic Longhorn, being less adaptable, was crowded out, not only in Egypt, but along the entire North African littoral. In other words, Brachyceros also accompanied the human stream of migration, along North Africa via Gibraltar into Europe and even into the Channel Islands where the Jersey represents the type. A part of the stream of advancing Brachyceros instead of entering Europe was diverted into West Africa as may be seen in several breeds of the West African Shorthorn. Du Toit (1927) and Stewart (1932) have referred to these cattle in Nigeria and the Gold Coast respectively.

At the present time, Brachyceros cattle are therefore not only to be found in North Africa and Europe, but also in their original Asia, where they occur in the northern part of the continent as far east as Japan.

While the movements described above were occurring, another invasion of North-east Africa took place, this time, the Semites entering mainly by way of South Arabia and Ethiopia and accompanied by their Longhorned Zebus.

Afrikander Cattle.

According to Epstein, cattle of the above type entered Africa about the end of the 3rd pre-Christian millenium. So great was their influence in Upper Egypt, chiefly on the Hamitic Longhorn, that (except for comparatively recent importations of the Shorthorned Zebu) the majority of all indigenous cattle south of the Sahara represents a cross of these two basic types.([5]) Besides the West

([4]) Neffgen (1904) states: " Die 'Kurzhornrasse', Tiere mit kurzen Hörnern, wie schon der Name sagt, kommt im 'Alten Reich' selten vor, dagegen mehr in späterer Zeit." A wall painting from Thebes (British Museum) showing this type is reproduced by several authors. See Kronacher's *Allgemeine Tierzucht* (1921—1. Abt., p. 169).

([5]) Called by Epstein " the Sanga ". As it is a cross, no further reference to it will be made here, the object of this study being an introduction of the three basic types.

African Shorthorn and the Mandingo cattle, the exception to the statement just made is the Afrikander of South Africa, which is the sole representative (in Africa) of the original Longhorned Zebu.(*)

The Shorthorned Zebu encountered to-day in Africa has been introduced within recent centuries and represents the influence in Asia of Brachyceros on the Longhorned Zebu. It must not be confused with the Sanga, which unfortunately is referred to by many African officials as Zebu.

Returning to the Afrikander, it is remarkable how in spite of several publications, oversea authors are frequently in doubt as to the origin of this breed. Snapp (1930), for example, describes the Afrikander as a cross of " these Kafir (i.e. Sanga type) cows . . . with Zebu or Brahma bulls for the purpose of obtaining an increase in size ".

The ancestor of the Longhorned Zebu is believed to be *Bos namadicus* which was first domesticated in the steppe country of Central Asia.

MAIN POINTS OF DIFFERENCE BETWEEN THE THREE BASIC TYPES OF AFRICAN CATTLE (EPSTEIN).

Feature.	Hamitic Longhorn. See Fig. 1.	West African Shorthorn. See Figs. 2 and 3.	Afrikander. See Figs. 4 and 5.
1. Head.....	" Comparatively short and broad."	Elongated frontal region which is concave.	Long and narrow. Forehead convex.
2. Horns.....	Long and upright. " Of same length and shape " in bull and cow. Base almost circular, substance " light coloured with dark tips, rarely dark throughout."	Short, fine, and dense in texture. Base circular.	Long and slender. Base oval. Horns leave head in lateral direction and show a moderate twist.
3. Dewlap...	" Only moderately developed."	Moderately developed.	Well developed and commencing at chin.
4. Hump....	" Not the slightest sign of a hump."	Not present.	Well developed and does not fall abruptly on to withers, but slopes gradually.
5. Height, at withers	" About 57 inches."	Varies from 3 feet to 4 feet.	About 5 feet.
6. Type of beast	Beef (" large framed beasts ").	? Milk. Generally a small beast.	Beef.

(*) Neffgen (1904) refers to the " hornlosen Tiere ", but not to the Longhorned Zebu.

Osteological Differences: Skull ([7]).

General Description.

Feature.	Hamitic Longhorn Bull. See Figs. 6, 7, and 8 (Epstein).	West African Shorthorn Bull (A. 82). See Figs. 9, 10, 11, and 12.	Afrikander Ox (A. 26). See Figs. 13, 14, and 15.
Frontal Surface.			
1. General shape...	"The head is comparatively short and broad."	Long, especially forehead, and fairly broad.	Long and comparatively narrow, especially between orbits.
2. Margin of orbit..	"The eye is big and prominent."	Slightly prominent.	Not prominent.
3. Profile..........	(Appears straight.)	Straight.	Convex.
Lateral Surface.			
4. Temporal fossa..	"The temple (temporal fossa) is broad and deep."	Deep, horizontal, and widely expanded caudally.	Deep and curved. Markedly influenced by base of horn.
5. Horn...........	"Lyre-shaped and slender." Upright.	Short and horizontal.	Lateral direction and slender with twist.
Basal Surface.			
Choanae...........	"The choanae begin a little more than ½ inch behind the third molar."	Choanae begin approximately 1 cm. in *front* of posterior edge of third molar.	As for Egyptian Longhorn.
Nuchal Surface.			
6. General.........	Separated from frontal surface by prominent and thick frontal ridge, which is level when viewed anteriorly and straight from side to side.	Frontal ridge thick but not prominent. From front it is convex (with central depression) and is straight from side to side.	Frontal ridge thick and prominent. From front it is convex and markedly curved from side to side.

Detailed Description.

Feature.	Hamitic Longhorn Bull (Epstein).	West African Shorthorn Bull (A. 82).	Afrikander Ox (A. 26).
Frontal Bone.			
Frontal surface— (*a*) caudal	"Rather flat. The frontals slope slightly down towards the temples."	Slightly concave. Lateral part slopes towards temporal region. Torus frontalis prominent.	Gently convex in all directions, especially towards horn cores.
(*b*) cranial.......	"A deep cavity between the orbital arches."	A deep saucer-like cavity.	A shallow depression being almost flat.

([7]) The skeleton of the West African Shorthorn was kindly presented to the Onderstepoort Museum by Capt. W. W. Henderson, M.R.C.V.S., Nigeria.

Feature.	Hamitic Longhorn Bull (Epstein).	West African Shorthorn Bull (A. 82).	Afrikander Ox. (A. 26).
(*c*) Interfrontal suture	"Ridge . . . a slight elevation" caudally, but "is hardly raised at all . . . slightly above the orbit."	No ridge-like prominence. A shallow furrow connects (*a*) and (*b*).	No special prominence along suture, but highest point of profile is central and situated between (*a*) and (*b*).
(*d*) Elevation limiting (*b*) caudally and laterally.	"Strong elevation."	Scarcely perceptible.	Scarcely perceptible.
(*e*) Supraorbital groove	"Begins at horn core and ends . . . 1 in. above nasals . . . deep and broad near upper portion of orbit . . . inner edge is sharp."	Begins at horn core and converges slightly towards nasals. Broad and sharp along inner edge.	Does not begin so close to horn core. The groves converge slightly towards nasals. Inner edge rounded and groove broad.
(*f*) Frontal ridge, from front	"Straight, except for a slight elevation towards the middle, which again shows a tiny depression in the centre."	Line slopes sharply upwards to Torus frontalis, whose summit is very slightly depressed.	Line slopes gently upwards to Torus frontalis which is not prominent. The summit is slightly depressed.
(*g*) From back.....	"Elevation appears much stronger. It is formed by the parietal and supraoccipital bones. Note lap-like extension of the parietals into the forehead"* (Torus frontalis).	Ridge while thick is not prominent. Parietals extend into Torus frontalis.	Ridge is thick, prominent and markedly curved (convexity cranial) in its length. Parietals extend into Torus frontalis.
Horn Core.			
(*h*) Neck...........	"Stalk-like process."	No stalk-like process.	Stalk well marked.
(*i*) Base...........	"Forms a pearled wreath which considerably exceeds in height" the neck. Almost circular.	Pearled wreath at base absent. Section of base is circular.	Pearled wreath at base absent. Section of base is oval.
(*j*) Furrows........	"Cores deeply furrowed."	Not well marked.	Well marked.
(*k*) Orbital arch....	"Rather thin and lower than the frontal elevations" (*d*).	Almost level.	Well developed and slightly higher than frontal elevations (*d*).
	"Higher (slightly) than" dorsal margin of temporal fossa.	Definitely higher.	Height of arch above dorsal margin striking.
(*l*) Fronto-lacrimal suture	General direction "straight" but edge "extremely jagged."	Direction straight and cranial half jagged.	Direction straight and entire edge jagged.

Feature.	Hamitic Longhorn Bull (Epstein).	West African Shorthorn Bull (A. 82).	Afrikander Ox (A. 26).
(*m*) Fronto-lacrimo-nasal point	A triangular hole . . . clearly distinguishable."	Triangular hole marked.	No sign of triangular opening.
Nasal Bone. (*n*) Frontal surface.	Missing from skull.	Profile slight concave.	Profile slightly convex.
Premaxilla. (*o*) Nasal process...	"Seems to have touched" nasal bone.	Does not meet.	In apposition.
Maxilla. (*p*) Palate.........	"Palate is very flat."	Arched from side to side.	Arched from side to side.
Occipital Bone. (*q*) Median occipital crest	"Squama . . . halved by a rough line running . . . from the upper border of the foramen magnum."	This crest, ventral to the external occipital protuberance, is very well marked.	Here the crest is scarcely perceptible.
Mandible. (*r*) Posterior edge of vertical ramus.	Bone missing, but Epstein notes "at right angles."	Forms obtuse angle with horizontal ramus.	Slightly greater than a right angle.

An important difference is seen in the spinous processes of the thoracic vertebrae. From the 6th vertebra onwards in the Afrikander (and indeed in all Zebus) not only is the upper 1/5th divided medially, but it is also compressed antero-posteriorly. In the West African Shorthorn no such cleft occurs. See Figs. 16 and 17.

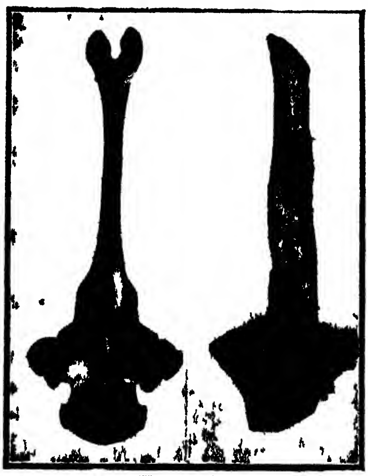

Fig. 16.

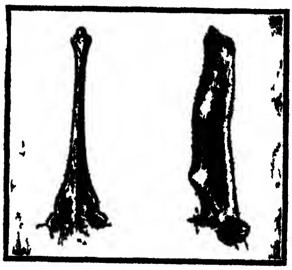

Fig. 17.

(Drawn by G. C Walker.)

Fig. 16.—Ninth dorsal vertebra of Afrikander cow (Curson). Compare with same vertebra West African Shorthorn bull (A.82).

Fig. 17.—Ninth dorsal vertebra of West African Shorthorn bull (A.82).

DISCUSSION.

In order that there may be no confusion, the detailed features are indicated on Figs. 13-15 of the Afrikander skull by the letters a—r.

The ages of the animals shown in the figures are approximately 3 years and 8 years for the West African Shorthorn and Afrikander respectively and not less than 5 in the case of the Hamitic Longhorn.

As will be observed from a study of the information tabulated above, the differences depend mainly on the development of the horns and the situation of the orbit.

It is not suggested that the above specimens are pure bred but they are sufficiently characteristic of the various types. It must be remembered that during the course of centuries much crossing has taken place and so all gradations are met with, but if typical skulls are selected, noteworthy differences are to be observed.

In a subsequent study it is hoped to give more information regarding measurements.

SUMMARY.

Assuming that the three foundation stocks of African cattle are the Hamitic Longhorn, Brachyceros, and Longhorned Zebu types, it is possible to differentiate between them (*a*) on account of their conformation, and (*b*) and because of differences in osteology. These differences are tabulated in the text and to emphasize them, photographs accompany the paper.

ACKNOWLEDGMENT.

We are indebted to the Directors of Veterinary Services of the following countries for assistance in connection with the compilation of this study: Union of South Africa (Dr. P. J. du Toit), Tanganyika (H. E. Hornby, Esq.), Nigeria (W. W. Henderson, Esq.), and Gold Coast (J. L. Stewart, Esq.). Prof. R. Dart of the University of the Witwatersrand kindly read the paper and made some useful observations.

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EXPLANATION OF FIGURES.

(Only Figs. 9-17 to scale, being approximately one-quarter of original.)

PHOTOGRAPHS ILLUSTRATING CONFORMATION (FIGS. 1-5).

Fig. 1.—Hamitic Longhorn bulls. (Taken from Kronacher's *Allgemeine Tierzucht,* 1 Abt., Fig. 73, from Adametz, 1921.)

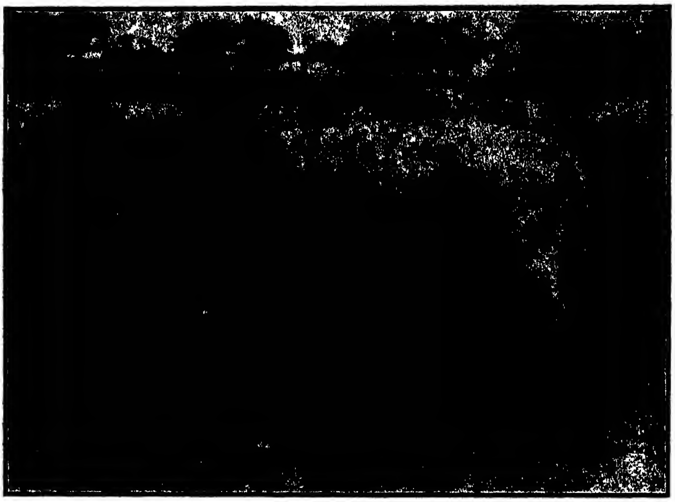


Fig. 2.—West African Shorthorn bull (photo provided by J. L. Stewart, M.R.C.V.S., P.V.O., Gold Coast, through H. E. Hornby, F.R.C.V.S., D.V.S., Tanganyika).

Fig. 3.—West African Shorthorn cow. (Photo taken by Dr. P. J. du Toit, D.V.S., Union of South Africa.)

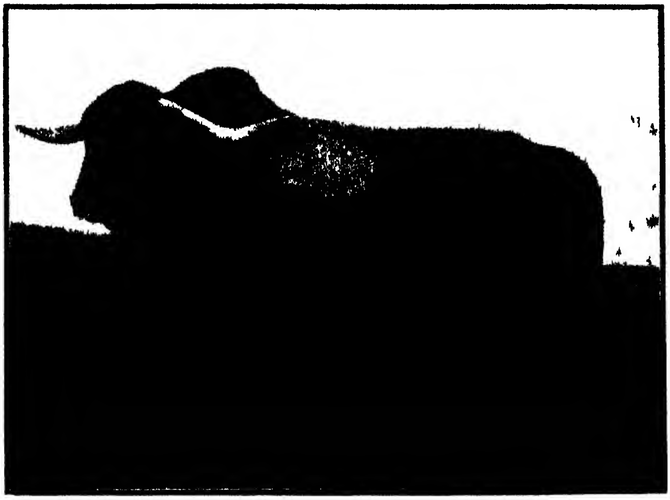


Fig 4 – Afrikander bull "Vaderland", bred by P. Biljoen, owned by Union Govt., purchased for £1,000 from Louis Wessels. (Photo provided by School of Agriculture Potchefstroom.)

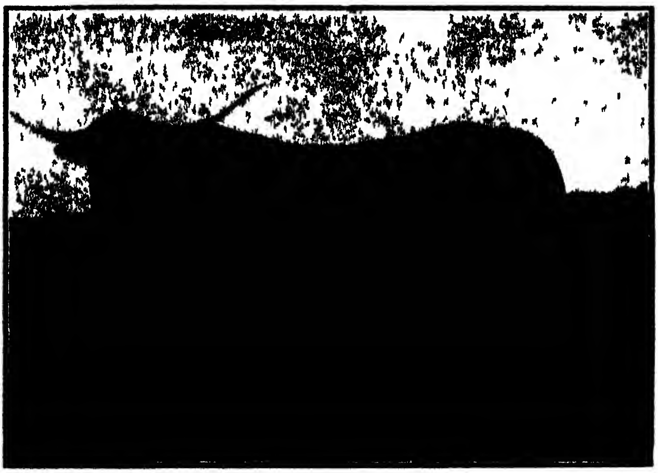


Fig. 5.—Afrikander cow "Lemoen", owned by P Biljoen, Hoopstad, O.F.S. (Photo provided by School of Agriculture, Potchefstroom.)

PHOTOGRAPHS INDICATING SKULL DIFFERENCES (FIGS. 6-15).

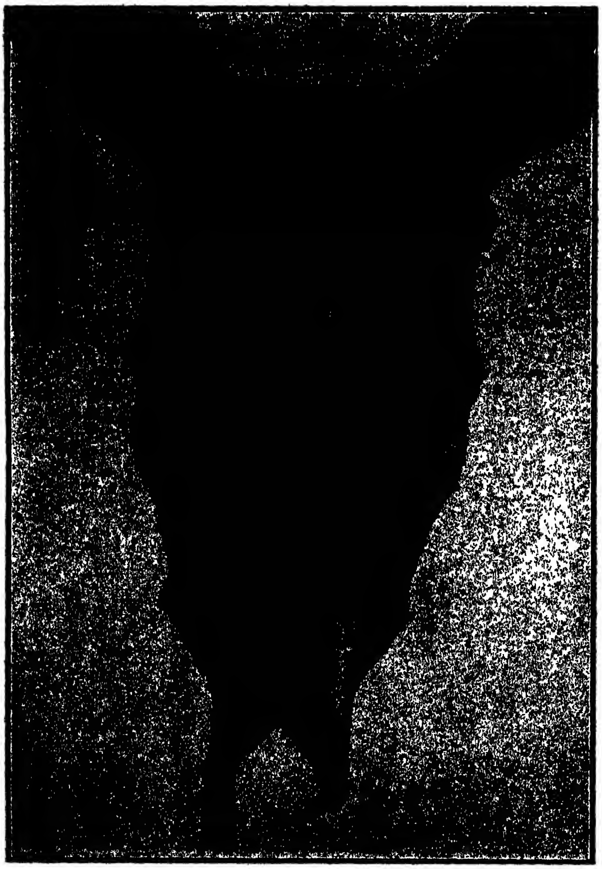


Fig. 6.—Frontal surface, Hamitic Longhorn bull.

Length of forehead, 234 mm.

Fig. 7.—Lateral surface, Hamitic Longhorn bull.

Fig. 8.—Nuchal surface, Hamitic Longhorn bull. Photographs 6-8 were furnished by Dr. H. Epstein, Welverdiend, Transvaal.

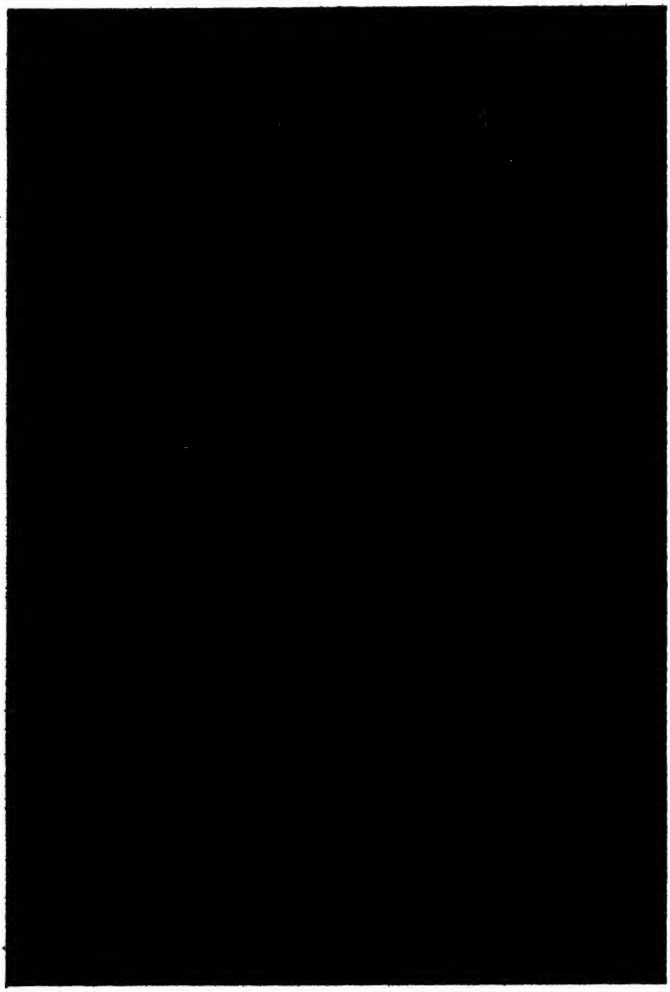
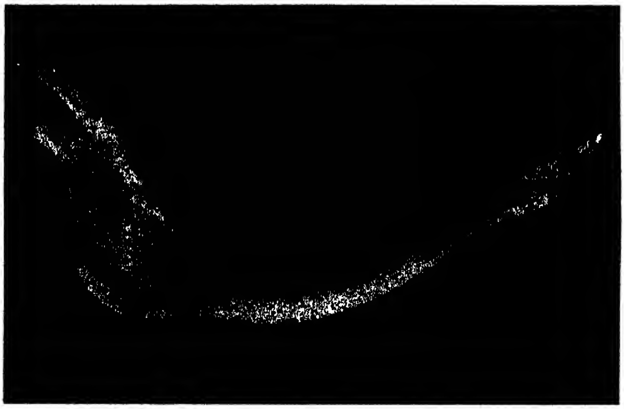


Fig. 9.—Frontal surface, West African Shorthorn bull (A.82).
Length of forehead, 193 mm.

Fig. 10.—Lateral surface, West African Shorthorn bull (A.82).



Figl. 11.—Mandible, West African Shorthorn bull (A.82).

Fig. 12.—Nuchal surface, West African Shorthorn bull (A.82). The skeleton was kindly presented to the Onderstepoort collection by W. W. Henderson, M.R.C.V.S., C.V.I., Nigeria, and photos were taken by T. Meyer.

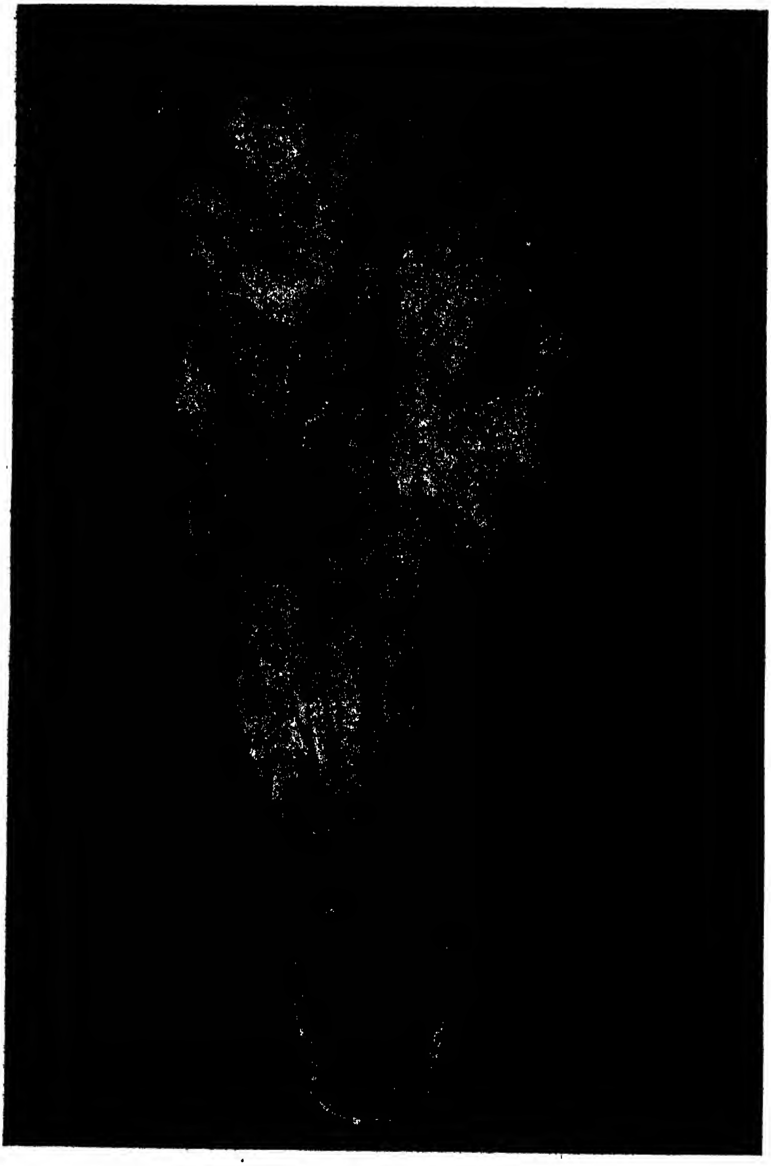


Fig. 13.—Frontal surface, Afrikander ox (A.26).
Length of forehead, 260 mm.

FIG. 14.

Fig. 14.—Lateral surface. Afrikander ox (A.26).

Fig. 15.—Nuchal surface, Afrikander ox (A.26). The skull was obtained through the kind offices of G. Pilditch, Pretoria Abattoirs. Photographs 9-15 by T. Meyer.

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SUBJECT INDEX, Vol. 3, Nos. 1 and 2.